GENETIC ANALYSIS OF GRAIN PROTEIN CONCENTRATION AND RELATED TRAITS IN THE ILLINOIS PROTEIN STRAIN RECOMBINANT INBRED POPULATION OF MAIZE

BY

CHRISTINE JEANETTE LUCAS

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2014

Urbana, Illinois

Doctoral Committee:

Professor Stephen P. Moose, Chair
Associate Professor Patrick Brown
Professor Sandra Rodriguez-Zas
Professor A. Lane Rayburn

Abstract

Maize kernels accumulate nitrogen supplied as amino acids by vegetative source tissues as the abundant zein proteins. However, zein protein is deficient in essential amino acids, and limited knowledge of zein regulation impedes approaches for achieving more nutritious Quality Protein Maize. We employ a candidate gene approach for investigating potential targets of more than a century of divergent selection for grain protein concentration in the Illinois High Protein (IHP) and Illinois Low Protein (ILP) strains. Selection for grain protein concentration has specifically altered α-zein protein abundance, but has also affected whole plant nitrogen metabolism. IHP exibits elevated N uptake, N assimilation and N remobilization from leaves to grain relative to ILP. IHP specifically hyperaccumulates the transport and storage amino acid, asparagine (Asn), in leaves and seeds. Additionally, grain protein and zein protein concentrations are subject to the maternal effect, where the progeny phenotype follows that of the maternal plant. This phenomenon has been observed in a variety of maize genotypes, including IHP and ILP, but its source is unknown.

A series of genetic resources derived from the Illinois selection experiment are particularly useful for investigating the genetic regulation of grain protein concentration and can additionally provide information about when candidate genes were targeted. These include inbreds derived from cycle 90, populations from cycles 65 and 100, and a population of recombinant inbred lines (Illinois Protein Strain Recombinant Inbreds or IPSRIs) created from the cross of IHP x ILP (cycle 70). Consistent with protein abundance, we document strong coordinate upregulated expression of all active alpha-zein genes in IHP seeds compared to ILP in inbreds and the cycles (65 & 105). We

demonstrate that genes important for regulating Asn-cycling and zein-synthesis pathways exhibit dramatic shifts in allele frequencies and gene expression during the Illinois selection experiment. We find that divergent fixation of expression variants in Asn-cycling genes occurred by cycle 65 of selection, and selection for more strongly-expressed alleles of both *Opaque2* and the *Prolamin-box factor* become important in more recent cycles of the Illinois Protein Strains. Using a GWAS approach on the IPSRI mapping population, we confirm the genetic effects of known gene candidates in Asn-cycling and alpha zein synthesis, and identify novel candidates.

Also included in these analyses is a novel phenotype that tracks 22-kD α-zein expression by use of a red fluorescent protein (mRFP1) promoter-reporter transgene, *Floury2*-mRFP1. This phenotype offers advantages to standard near infrared reflectance (NIR) spectroscopy methods for measuring kernel composition in that it specifically tracks α-zein expression. As a result, this phenotype more readily detected candidate genes annotated as regulatory variants. This phenotypes was also used in a series of reciprocal crosses to investigate possible mechanisms underlying the maternal inheritance of grain protein concentration. These studies provided strong evidence for the role of plant nutrient status as the primary mechanism, which could be explained by the activities of enzymes in the Asn-cycling pathway within vegetative tissues. Collectively, this knowledge will assist breeding for QPM and reveal evolutionary features of regulatory variation. Due to the large impact of selection on nitrogen metabolism, it may also lead to improvements in nitrogen utilization in maize and possibly other cereals.

Acknowledgements

I gratefully acknowledge the College of ACES, the Department of Crop Sciences, and Stephen Moose's laboratory for enabling my studies through financial support. These have included various fellowships, awards and assistantships throughout my degree, as well as the financial means to attend conferences. I gratefully acknowledge the Department of Crop Sciences staff and faculty for providing an excellent educational experience at not only one of the best universities in the country, but one that is known for its agricultural roots. I would like to thank my committee members for serving on my committee, for their invaluable feedback, and for helping me to troubleshoot when needed. I would also like to thank Shiv Sivaguru at the Institute for Genomic Biology and members of the Ed Buckler laboratory at Cornell University, especially Rob Elshire, for successful collaborations. I thank my colleagues and friends for many, many intellectual exchanges that have taught me to be a better researcher and also for the cultural exchanges they have provided. I thank my family members for their constant support and listening, but especially my Dad for his many words of wisdom and encouragement. Finally, I would like to thank my adviser, Steve Moose, for the opportunity to conduct research on one of the longest running genetics experiments in plants using the latest genomics technologies, and to prepare me for a career in the seed industry. I gratefully acknowledge his dedication to teaching, sound research methods, and constant support and encouragement. Thank you!

Table of Contents

CHAPTER 1. LITERATURE REVIEW: ZEIN PROTEIN AND THE ILLINOIS LONG-TERM SELECTION EXPERIMENT FOR KERNEL COMPOSITION TRAITS

Zein Protein

Cereal crops provide over 50% of the world population's calories, 65% if we consider calories consumed from the animal products derived from livestock fed cereal grain (Godwin et al. 2009). However, cereal grain is regarded as nutritionally poor because grain protein is deficient in several amino acids essential to human and livestock consumption, including lysine, tryptophan, threonine and methionine. For example, maize proteins contain less than 3% of lysine, only about half of the 5.5% recommended by the Food and Agriculture Organization for human nutrition (Huang et al. 2004). For this reason, there has been a great effort to improve the nutritional composition of cereal grain.

During maize seed development, nitrogen from plant vegetative tissues (source) is mobilized into the kernel (sink) where it is used to synthesize endosperm storage proteins that become the nitrogen source for the developing seedling. The majority of storage protein assembles into protein bodies and accumulates in the subaleurone and starchy endosperm. Endosperm storage proteins constitute 70% of total kernel protein with approximately 50 -60% belonging to the alcohol-soluble, proline- and glutamine-rich group of storage proteins, the prolamins (Tsai, 1990). Maize prolamins, collectively termed zeins, are the most abundant endosperm storage protein, but lack the previously mentioned essential amino acids, making them nutritionally poor. Therefore, an important goal of maize breeders worldwide is to actually reduce zein accumulation (Prassana, 2001). Due to the inverse relationship between grain protein and starch concentration, and the strong positive correlation between starch concentration and

yield, reducing protein concentration has additional opportunities for increasing grain yield.

Zein proteins can be classified into four large multigene families based on differential solubility and polypeptide compositions: 14 kD β- zeins, 27 and 16 kD γ- zeins and 10 and 18 kD δ- zeins, and the 19 and 22 kD α- zeins (Esen 1986). The α- zein gene family accounts for the majority of the total prolamin fraction of the endosperm and is encoded by an estimated 110 to 130 gene members mapped to five regions in the genome, 4L, 4S, 7S, 10L and near the centromere of chromosome 1 (Viotti et al., 1979). Genes encoding the α- zein gene family can be further resolved into gene subfamilies according to sequence homology and copy number based on DNA hybridization data as a classification scheme (Song and Messing, 2002; Woo et al. 2001). Subfamilies z1A, z1B, z1D range in molecular weight from 19-21 kD (19kD) and z1C from 22-23kD (22kD) (Shewry and Casey, 1999), where observed variation in molecular weight occur due to internal insertions and deletions of the original gene (Heidecker et al., 1991).

Recent analysis of gene collinearity and sequence divergence of prolamins in members of the *Poacea* family (Xu and Messing 2008, Xu and Messing 2009) showed that the α-zeins are the youngest gene family, arising only after the divergence of the *Panicoideae* subfamily (maize, sorghum, little millet) from *Ehrhartoideae* (rice) and *Pooideae* (barley, wheat and brachypodium) subfamilies. In addition to their recent evolution, the α-zeins continue to evolve at a high rate. Compared to the relatively rare tandem duplications resulting from polyploidization in older zein gene families (β, γ, and δ), the α-zeins have incurred a high frequency of tandem duplication events following

dispersal to different genomic locations. The net result is the formation of compact clusters of multiple gene copies that also contain intergenic transposon-derived repeats. For example, the α-zeins have been sequenced in inbred varieties B73 and BSSS53, and the collective results show a total of 41 and 48 genes in B73 and BSSS53, respectively, which are tightly clustered within five small groups of tandemly repeated genes on three chromosomes (Song et al. 2001, Song and Messing 2002, Miclaus, Xu and Messing 2011). In inbred variety BSSS53, 22 z1C gene copies span a region of only 168 kb with the *Floury2* locus residing approximately 20cM away (Song et al. 2001). This type of organization has favored the production of significant copy number variation and non-collinearity among maize inbred lines (Song and Messing 2003), likely resulting from unequal crossing over and possibly contributing to regulatory variation. For example, one study identified 16 non-allelic and 11 allelic α-zeins shared between B73 and BSSS53; 8 non-allelic and 14 allelic genes between B73 and W22; and 18 non-allelic and 10 allelic genes between BSSS53 and W22 (Feng et al 2009). Haplotype characterization for all α-zein subfamilies has been reported by Song and Messing (2003) and Miclaus et al. (2011).

Zein mRNA Expression

The high degree of presence absence variation and non-collinearity of α-zein genes may contribute to observed expression variation among inbred maize lines. Not only do inbred lines exhibit significant differences in expressed gene sets, but even allelic genes are not uniformly expressed at the same level (Consoli and Damerval 2001, Song and Messing 2003, Feng et al. 2009, Miclaus et al. 2011, Song et al. 2001).

Additionally, only a fraction of α-zein gene coding sequences produce transcripts, indicating the presence of pseudogenes interspersed with functional genes (Marks et al., 1995; Woo et al., 2001). For example, only 7 of the 23 22kD α-zeins are expressed in BSSS53, including the *Floury2* allele (Song 2001). Variation in peak expression time has also been reported for each subfamiliy; according to Feng et al., who reported peak expression for z1A at 18 DAP, z1B at 22 DAP and z1C and z1D at 24 DAP (2009).

Despite differences in expression due to genotype or gene family, several less subtle patterns in α-zein expression suggest coordinated transcriptional regulation throughout development. Regardless of genotype, expression begins around 10 days after pollination (DAP) and continues throughout grain fill. Collectively, these results suggest the role of common regulatory factors with a possibility for distinct factors that function to fine-tune expression by controlling subsets of gene copies. What is apparent is that α-zein regulation is complex and likely occurs at multiple levels.

Factors Influencing Grain Composition

Despite 40 years of breeding for Quality Protein Maize (QPM) and intense efforts directed at characterizing α-zein regulation, the genetic regulators of grain protein and α-zein content still remain largely unknown. One known regulatory factor is the PROLAMIN-BOX FACTOR (PBF), a Dof-class transcription factor (VicenteCarbajosa et al., 1997) that specifically accumulates during endosperm development in a manner that is consistent with it playing a major role in the coordinated transcription of all zein genes (Marzabal et al. 2008). PBF has been shown to bind a highly conserved sequence

element termed the “prolamin box” in all zein genes, as well as prolamin genes from other cereal species, including sorghum, wheat and barley (Forde et al. 1985).

Another known transcriptional regulator is the bZIP class transcription factor, OPAQUE2 (O2), which binds a sequence element present only in the promoters of the 22-kD α-zein and 15-kD β-zein genes, but not other zein genes (Schmidt et al., 1992). Genes encoding bZIP proteins with functions related to O2 have been identified in wheat, barley, coix and sorghum. The O2 binding site in maize is only 20 nucleotides downstream from the P-box, and the PBF and O2 proteins have been shown to interact with each other *in vitro* to increase zein gene expression in an additivie fashion in transient expression assays in wheat and rice (Hwang et al., 2004). The presence of both universal and distinct regulatory factors, as exemplified by PBF and O2, respectively, may represent one method for achieving subtle variation in zein expression without affecting overall patterns of coordinated transcriptional regulation.

In addition to transcriptional regulation, interactions among zein proteins and protein folding and assembly in the maize endosperm can also influence zein accumulation by facilitating the assembly of zeins into protein bodies (Bagga et al., 1997; Fontes et al., 1991; Holding et al., 2007). One known factor is a binding immunoglobulin protein (BiP), whose biological function is to mediate protein folding and assembly in maize endosperm (Fontes et al., 1991).

Total and zein protein concentration are also subject to what is known as the maternal effect, where the progeny phenotype follows that of the maternal genotype. This observation is well documented in maize, where it has been observed in the progeny of crosses between a number of genotypes (Letchworth and Lambert 1998),

including reciprocal crosses between IHP and ILP, as well as crosses between these genotypes with elite inbred varieties (Reggiani, Brambilla and Bertani, 1985; Tsai, Dweikat and Tsai, 1990; Tsai and Tsai, 1990). At least three possible mechanisms have been proposed for the strong maternal effects on grain protein concentration (Moose et al., 2004), including endosperm dosage effects, maternal imprinting of zein gene expression, and nutrient supply from vegetative source tissues (Balconi et al. 1993). The contribution of each of these mechanisms is investigated in the subsequent chapters.

Breeding for Quality Protein Maize (QPM)

The discovery of several mutations in *Opaque2* (*o2*) and *Floury2* (22kDα) (*fl2*) that lead to a reduction in 22-kD α-zein accumulation and a corresponding increase in the more nutritionally balanced lysine-rich non-zein endosperm storage proteins was a significant step towards achieving QPM. For instance, *o2* reduced zein accumulation by 65% in the inbred variety W64A, which corresponded to a 250% increase in soluble lysine (Hunter et al., 2002). A 700% increase was observed in inbred variety Oh43 (Azevedo et al., 2003). However, a large degree of phenotypic variation was observed in a survey of 93 inbreds, and levels were still below FAO dietary recommendations (Gloverson et al., 1996). Furthermore, phenotypic modification associated with QPM is plagued by reduced yield and a softer opaque endosperm due to altered starch structure (Gibbon et al., 2003), which makes kernels susceptible to insect and mechanical damage and subsequent microbial infection.

Attempts to overcome undesirable agronomical traits associated with the *o2* mutation have been partially successful, but not straightforward. The combination of the starch-modified *su2* mutant with *o2* successfully produced a denser kernel, but also reduced grain yield even more. Conventional breeding for a more vitreous kernel has helped to remedy the softer endosperm phenotype, but introgression of the *o2* mutation into elite inbred lines is complicated due to its recessive nature. Using a rapid line conversion strategy with a two-generation backcross program, Babu et al. (2005) achieved high protein quality and hard endosperm characters. However, low yield associated with the *o2* mutation has been especially difficult to overcome; breeding for high-yielding *o2* hybrids only raised yield to about 90% of normal counterparts (Tsai 1989). Additionally, O2 has been shown to regulate other non-target genes, including *b-32* ribosome-inactivating protein (Lohmer et al., 1991), *orthophosphate dikinase* (Maddaloni et al., 1996), and a number of other genes (Hunter et al., 2002). Therefore, alternative methods for achieving QPM are sought, which necessarily requires knowledge of additional regulators.

Measuring Kernel Total N and Zein Protein Concentration

Several analytical procedures are available for measuring kernel composition traits. One direct analytic method for measuring %N is combustion analysis (CE Elantech Inc. NA2000 N-Protein, Lakewood, NJ) using the method of Dumas (Kirsten and Hesselius, 1983). An indirect analytic method is Near Infrared Reflectance (NIR) spectroscopy, which measures the percent of amine chemical bonds. NIR relies on the development of robust calibration(s) for the trait(s) of interest, which are typically

created using N combustion. With respect to measuring protein concentration, it is important to note that the above methods actually measure total N. However, to maintain consistent with the literature, total N is referred to as protein concentration in this body of work with the acknowledgement that the methods described herein cannot separate bound from free amino acids, nor can they differentiate between different types of proteins.

Choice of method depends on the allocated time and cost, as well as the necessity of preserving intact grain. While combustion analysis is a direct analytic method, it requires destruction of the grain sample, can be expensive, and requires an additional sample for separate oil analyses, which can be assayed using Nuclear Magnetic Resonance (NMR). NIR is an indirect analytic method that uses linear regression to estimate unknown samples based on a calibration built with known samples. However, the estimation can be quite accurate with a comprehensive calibration, and the calibrations for the instruments used here were developed using direct methods. N combustion analysis was used for generating the protein calibration. Approximately forty grain samples from the Illinois Protein and Oil Strains were used to develop a NIR calibration for the DICKEY-John instrument in 2006. The samples ranged in protein from 4.4% to 28.74%, oil from 1.5% to 20.4%, and moisture from 9-10%. Significantly deviant estimations are only likely to occur if predictions lie outside the range of the calibration, and updates to the calibration are recommended on a yearly basis.

The advantages of NIR over N combustion are that it provides simultaneous estimations of protein, starch, and oil, which eliminates the need for multiple methods.

Added benefits of more recent instrument models are that they may not require sample destruction and can measure other components where calibrations have been established. However, N combustion is more accurate because it is a direct method. Both NIR and N combustion methods were used here for measuring total N.

Alternative Measures of Zein Protein Accumulation

While NIR remains the standard method for measuring kernel composition traits, it can neither differentiate between free amino acids and bound protein, nor between different types of protein. Here, where it is of interest to understand the regulation of a specific type of protein, zein, NIR may be too coarse a phenotype. Alternatively, the zein fraction can be extracted and quantified using the BCA assay or SDS-PAGE, but these methods are labor intensive and require tissue destruction. Additionally, the BCA assay cannot separate gene products specific to individual zein subfamilies, and while SDS-PAGE can overcome this, it is only semi-quantitative.

Due to the strong correlation between zein protein and zein expression (Lucas et al., 2013), zein expression can serve as a substitute for zein protein abundance. However, even approaches for studying zein gene expression are complicated, owing to their high copy number, high sequence homology, and the presence of pseudogenes. The use of reporter genes may help overcome this problem; when fused to a zein gene promoter, the expression of the reporter gene can be measured in a rapid, quantitative, and non-destructive manner to estimate expression of the zein gene of interest. Fluorescent proteins, such as green fluorescent protein (GFP) from jellyfish, modified derivatives of GFP with different spectral properties, and the monomeric red fluorescent

protein from reef coral (DsRed), have gained popularity as reporter genes in plants because they have overcome some limitations associated with other reporter genes, such as GUS, which is difficult to measure accurately and requires tissue destruction.

To track α-zein expression, the studies described here make use of a modified DsRed fluorescent protein (mRFP1) (Campbell et al., 2002) fused to a single 22-kD α-zein gene, *Floury2* (Mohanty et al., 2009). When backcrossed to the inbred Illinois Protein Strains, *Floury2*-mRFP1 expression closely followed that of endogenous 22-kD α-zein gene expression and was highly correlated with protein and α-zein concentration (Lucas et al., 2013). Additionally, *Floury2*-mRFP1 gene expression can be rapidly monitored during seed development and quantified by direct imaging, and does not require sample destruction like with NIR or N combustion. A precise method for quantifying *Floury2*-mRFP1 expression has been developed and is described in chapter three as an alternative to NIR. The resulting phenotype is used in a series of experiments to investigate the maternal inheritance of grain protein concentration and in marker-trait association studies. Additionally, because this phenotype tracks α-zein expression, it may more readily identify regulatory variants.

Germplasm Resources Derived from the Illinois Selection Experiment

Here we use genetic resources derived from the Illinois long-term selection experiment, the world's longest running continuous genetics experiment in higher plants, to study the genetic regulation of grain protein in maize. The objective of the experiment when initiated in 1896 was to determine if kernel protein and oil concentrations could be altered by selective breeding, which became apparent after

only ten cycles of selection. The objective was revised to determine the limits of artificial recurrent selection. Parallel experiments conducted for either kernel protein or kernel oil concentrations have produced 12 distinct Illinois Protein or Oil Strains that represent more than 800 instances of phenotypic selection from a common open-pollinated variety, Burr's White (Moose et al., 2004). One-hundred ten cycles of divergent recurrent selection have altered grain protein from an average of 12% to over 32% protein in Illinois High Protein (IHP) and to only 4% in Illinois Low Protein (ILP). After 48 cycles of forward selection in IHP and ILP, the direction of selection was reversed to generate Illinois Reverse High Protein (IRHP) and Illinois Reverse Low Protein (IRLP), which contain 5% and 18% grain protein concentration, respectively (Dudley and Lambert, 1992, Moose et al., 2004). Collectively, these strains are referred to as the Illinois Protein Strains (IPS). Mean protein concentrations over the course of the experiment are plotted in Figure 1.1. The parallel selection experiment for kernel oil concentration did not significantly alter protein concentration, as illustrated by Illinois High Oil (IHO) and Illinois Low Oil (ILO), which serve as unselected controls for the kernel protein selection experiment.

In addition to the populations, several genetic resources from this experiment were created that are particularly well-suited to genomics applications, including inbred lines derived from cycle 90 of the selection experiment that represent the phenotypic variation in the selected strains, populations of individuals from cycles 65 and 105 for comparisons spanning a 40 year period, and a recombinant inbred mapping population created by crossing individuals from cycle 70 of IHP and ILP, followed by seven generations of random mating (Dudley et al., 2007) and six generations of inbreeding

(Moose laboratory) (Lucas et al., 2013) (Figure 1.1). This latter resource is referred to as the Illinois Protein Strain Recombinant Inbred (IPSRI) population. The IPSRIs consist of 500 individuals derived from 200 RM7S2 families. A clustering analysis was conducted on the 500 individuals using 500 SNP markers (Monsanto, unpublished data) based on Euclidean distance coefficients and the unweighted Pair Group Method with Arithmetic Mean (UPGMA) as an option (Sokal and Michener, 1958). From this analysis, 138 individual clusters were identified containing 1-5 individuals each. One individual was chosen randomly from each clade to eliminate closely related individuals and reduce population structure. This resulted in a population of 138 IPSRIs. The phenotypic distribution of the reduced set (n=138) spans the distribution of the full set (n= 500), which ranges from 11-21% total N, and captures the majority of phenotypic variation in the IHP and ILP populations at cycle 70 (Figure 1.1)(Lucas et al., 2013).

Biochemical analysis of the IPS throughout the selection experiment revealed that changes in grain protein concentration resulted primarily from changes in accumulation of α-zeins (Bhattramakki, Sachs and Kriz, 1996). Therefore, the populations derived from the IPS provide an ideal genetic background for characterization of α-zein gene regulation with implications for improving QPM.

Previous Attempts to Identify Grain Composition QTL

The physiological extent to which divergent selection has altered whole plant N and C metabolism in the IPS has led to the hypothesis that many genes have also been affected (Moose et al., 2004). This hypothesis is supported by continued progress of the strains after over 100 cycles of recurrent selection (Dudley and Lambert, 2004),

which illustrates the quantitative nature of these traits. Several genetic mapping studies have identified quantitative trait loci (QTL) influencing protein (Goldman et al., 1993; Dijkhuizen et al., 1998; Dudley et al., 2004; Clark et al., 2006; Dudley et al., 2007) and oil concentrations (Clark et al., 2006) utilizing populations created by crossing individuals from cycle 70 of IHP and ILP (IPSRIs before inbreeding), or IHO and ILO (Dudley et al., 1977). The results of these studies suggest the presence of many QTLs having small phenotypic effects, which is consistent with theoretical estimates for the number of effective genetic factors based on genetic variances of 102 to 178 genetic factors for protein and 14 to 69 factors for oil (Dudley and Lambert, 1991; Dudley et al., 2004). Similar results were found using other maize germplasm. For example, 21 QTL were identified for starch concentration, 26 for protein and 22 for oil by a combination of joint-linkage QTL mapping and genome-wide association studies using the nested association mapping and inbred association panels (Cook et al., 2012). Another study that used a RIL population created by the cross of an American Iodent line and a French semident line also detected numerous QTL for protein and starch (Sene et al., 2001).

The precision of quantitative trait loci mapping can be limited by the large size of linkage blocks, depending on population structure. Advanced intermated populations can overcome this problem due to increased recombination frequency and precision of associations between markers and QTL and between QTL. This has been demonstrated in maize with the intermated B73 x Mo17 recombinant inbred line (IBMRIL) population, which has undergone five cycles of random mating to increase recombination before developing inbred lines. This was shown to increase the genetic

map distance by four-fold and increase resolution (Lee et al., 2002). In order to reduce linkage disequilibrium that was predicted to increase following forward selection in the Illinois selection experiment, the progeny of the IHP x ILP and IHO x ILO crosses were random mated by Dudley (1994). Analysis of populations following seven generations of random mating (RM7) showed that this strategy was successful in reducing linkage disequilibrium (LD) and breaking up coupling-phase linkages among genes controlling protein concentration, as assessed in crosses of the RM7 population to two common testers (Dudley, 1994; Dudley et al., 2004). Ten generations of random mating following the cross of IHO x ILO produced similar results (Clark et al., 2006). Additionally, Dudley et al. (2004) showed large reductions in the percentage of markers declared significant between the Syn0 (one generation of random mating the F1) and Syn4 (four generations of random mating the F1) populations of IHP x ILP. Similar results were observed using populations derived from the cross of Illinois High Oil (IHO) x Illinois Low Oil (ILO) (Willmot et al., 2006; Clark et al., 2006). Thus, a major advantage of using the random-mated populations, such as the IPSRIs, for purposes of genetic mapping experiments is the increased recombination frequency, which will allow for more precise associations between markers and QTL.

Research Objectives

Improvement of the nutritional quality of maize grain remains an important objective of maize breeders worldwide, owing in part to poor understanding of α-zein regulation. Better characterization of α-zein regulation may additionally elucidate mechanisms for prolamin reduction in other cereals, including sorghum (kafirins), wheat

(gliadins), barley (hordeins), and rice (oryzins). In addition, the strong inverse relationship between protein concentration in cereal grains and both endosperm starch and grain yield may simultaneously facilitate identification of regulatory variants controlling starch concentration and yield.

This project leverages more than a century of recurrent divergent selection for grain protein concentration in Illinois' signature selection experiment, which has produced the known phenotypic extremes for α-zein gene expression and α-zein protein accumulation in the Illinois High Protein (IHP) and Illinois Low Protein (ILP) strains. Additional genetic resources derived from this experiment (inbreds, cycles 65 & 105 and the IPSRIs) are particularly well-suited to genomics applications. Furthermore, we have recently developed a novel phenotype for association and mapping studies that involves a DsRed fluorescent protein reporter, mRFP1, fused to the regulatory sequences from a single α-zein gene, *Floury2*. Because the reporter gene specifically tracks expression of α-zein, rather than other components of total N, it provides additional advantages for identification of regulatory variants of α-zein gene expression.

The objectives of this research are as follows: **1.)** To investigate the source of the maternal effect on grain protein concentration, **2.)** To investigate the roles of candidate genes involved in both zein synthesis and Asn-cycling pathways **3.)** To develop a method for quantifying *Floury2*-mRFP1 expression, and **4.)** To identify genomic regions associated with kernel composition traits, including phenotypes collected using NIR or N combustion and the novel *Floury2*-mRFP1 phenotype, using genome-wide SNP-trait association studies and QTL mapping approaches. This knowledge will assist breeding for maize improvement and reveal evolutionary features of regulatory variation.

Figure

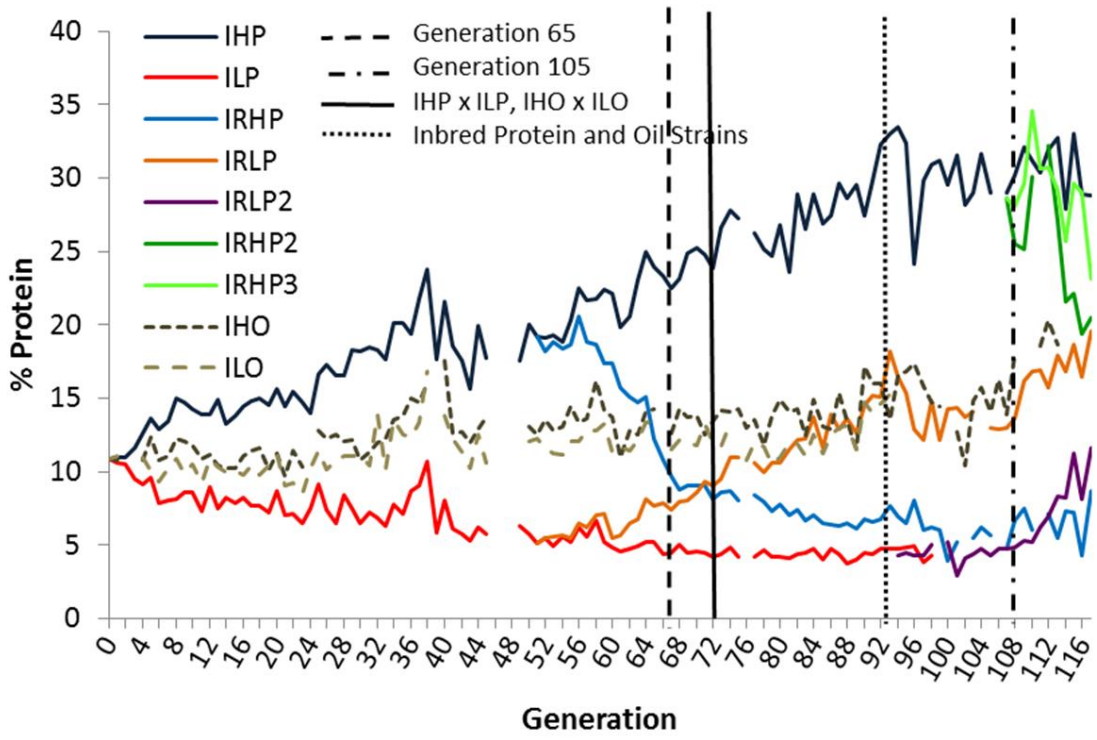


Figure 1.1. *From Lucas et al., 2013.* Selection responses in the Illinois Protein Strains. Protein concentrations for IHO and ILO are plotted as dashed lines. The vertical lines highlight important generations of the experiment, generation 65 for which oldest seed is still available, generation 70 from which QTL mapping populations have been derived from crosses of selected strains, generation 90 as a source of inbred lines derived from the Illinois Protein Strains, and generation 105 for which comparisons are made with generation 65.

References

Azevedo, R., Damerval, C., Landry, J., Lea, P.J., Bellato, C.M., Meinhardt, L.W., Guilloux, M.L., Delhaye, S., Toro, A.A., Gaziola, S.A. and Berdejo, B.D.A. (2003) Regulation of maize lysine metabolism and endosperm protein synthesis by opaque and floury mutations. *European Journal of Biochemistry.* 270 (24): 4898-4908.

Babu R, Nair SK, Kumar A, Venkatesh S, Sekhar JC, Singh NN, Srinivasan G, Gupta S: Two-generation marker-aided backcrossing for rapid conversion of normal maize lines to quality protein maize. Theories of Applied Genetics 2005, 111: 888–897.

Bagga, S., H. P. Adams, F. D. Rodriguez, J. D. Kemp & C. SenguptaGopalan (1997) Coexpression of the maize delta-zein and beta-zein genes results in stable accumulation of delta-zein in endoplasmic reticulum-derived protein bodies formed by beta-zein. *Plant Cell,* 9**,** 1683-1696.

Balconi, C., E. Rizzi, M. Motto, F. Salamini & R. Thompson (1993) The Accumulation of Zein Polypeptides and Zein Messenger-Rna in Cultured Endosperms of Maize Is Modulated by Nitrogen Supply. *Plant Journal,* 3**,** 325-334.

Bhattramakki, D., M. M. Sachs & A. L. Kriz (1996) Expression of genes encoding globulin and prolamin storage proteins in kernels of Illinois long term chemical selection strains. *Crop Science,* 36**,** 1029-1036.

Campbell, R., Tour, O., Palmer, A., Steinbach, P., Baird, G., Zacharias, D., and Tsien, R. (2002) A monomeric red fluorescent protein. *PNAS.* 99 (12): 7877-7882.

Clark, R. M., T. N. Wagler, P. Quijada & J. Doebley (2006) A distant upstream enhancer at the maize domestication gene tb1 has pleiotropic effects on plant and inflorescent architecture. *Nature Genetics,* 38**,** 594-597.

Coleman, C., and Larkins, B. The prolamins of maize. Eds. P. Shewry and R. Casey. Dordrecht: Kluwer Academic Publishers, 1999. 109-139.

Consoli, L. & C. Damerval (2001) Quantification of individual zein isoforms resolved by two-dimensional electrophoresis: genetic variability in 45 maize inbred lines. *Electrophoresis,* 22**,** 2983-9.

Dijkhuizen, A., J. W. Dudley, T. R. Rocheford, A. E. Haken & S. R. Eckhoff (1998) Near-infrared reflectance correlated to 100-g wet-milling analysis in maize. *Cereal Chemistry,* 75**,** 266-270.

Dudley, J. W. 1977. Seventy-six generations of selection for oil and protein percentage in maize. In *Proc. Intl. Conf. on Quantitative Genetics,* ed. O. K. E. Pollak, and T.B. Bailey, Jr. , 459-473. Iowa State University Press.

Dudley, J. W. (1994) LINKAGE DISEQUILIBRIUM IN CROSSES BETWEEN ILLINOIS MAIZE STRAINS DIVERGENTLY SELECTED FOR PROTEIN PERCENTAGE. *Theoretical and Applied Genetics,* 87**,** 1016-1020.

Dudley, J. W., D. Clark, T. R. Rocheford & J. R. LeDeaux (2007) Genetic analysis of corn kernel chemical composition in the random mated 7 generation of the cross of generations 70 of IHP x ILP. *Crop Science,* 47**,** 45-57.

Dudley, J. W., A. Dijkhuizen, C. Paul, S. T. Coates & T. R. Rocheford (2004) Effects of random mating on marker-QTL associations in the cross of the Illinois High Protein X Illinois Low Protein maize strains. *Crop Science,* 44**,** 1419-1428.

Dudley, J. W. & R. J. Lambert (1992) 90-Generations of Selection for Oil and Protein in Maize. *Maydica,* 37**,** 81-87.

Feng, L. N., J. Zhu, G. Wang, Y. P. Tang, H. J. Chen, W. B. Jin, F. Wang, B. Mei, Z. K. Xu & R. T. Song (2009) Expressional profiling study revealed unique expressional patterns and dramatic expressional divergence of maize alpha-zein super gene family. *Plant Molecular Biology,* 69**,** 649-659.

Fontes, E. B. P., B. B. Shank, R. L. Wrobel, S. P. Moose, G. R. Obrian, E. T. Wurtzel & R. S. Boston (1991) Characterization of an Immunoglobulin Binding-Protein Homolog in the Maize Floury-2 Endosperm Mutant. *Plant Cell,* 3**,** 483-496.

Forde, B. G., A. Heyworth, J. Pywell & M. Kreis (1985) Nucleotide sequence of a B1 hordein gene and the identification of possible upstream regulatory elements in endosperm storage protein genes from barley, wheat and maize. *Nucleic Acids Research,* 13**,** 7327-39.

Gibbon BC, Wang X and Larkins BA: Altered starch structure is associated with endosperm modification in Quality Protein Maize (2003) *PNAS*. 100 (26): 15329-15334.

Gloverson, L., J.E. Moro, E. Habben, B.R. Hamaker, and B.A. Larkins. (1996) Characterization of the variability in lysine content for normal and opaque2 maize endosperm. *Crop Science*. 36(6):1651-1659.

Godwin, I. and Izquierdo, L. (2005) Molecular Characterization of a Novel Methionine-Rich δ-Kafirin Seed Storage Protein Gene in Sorghum (*Sorghum bicolor* L.). *Cereal Chemistry.* 82 (6): 706-710.

Goldman, I. L., T. R. Rocheford & J. W. Dudley (1993) Quantitative trait loci influencing protein and starch concentration in the Illinois Long-term selection maize strains. *Theoretical and Applied Genetics,* 87**,** 217-224.

Heidecker, G., Chaudhuri, S., Messing, J. (1991) Highly clustered zein gene sequences reveal evolutionary history of the multigene family. *Genomics.* 10 (3): 719-732.

Holding, D. R., M. S. Otegui, B. L. Li, R. B. Meeley, T. Dam, B. G. Hunter, R. Jung & B. A. Larkins (2007) The maize floury1 gene encodes a novel endoplasmic reticulum protein involved in zein protein body formation. *Plant Cell,* 19**,** 2569-2582.

Huang, S. S., W. R. Adams, Q. Zhou, K. P. Malloy, D. A. Voyles, J. Anthony, A. L. Kriz & M. H. Luethy (2004) Improving nutritional quality of maize proteins by expressing sense and antisense zein genes. *Journal of Agricultural and Food Chemistry,* 52**,** 1958-1964.

Hunter, B. G., M. K. Beatty, G. W. Singletary, B. R. Hamaker, B. P. Dilkes, B. A. Larkins & R. Jung (2002) Maize opaque endosperm mutations create extensive changes in patterns of gene expression. *Plant Cell,* 14**,** 2591-2612.

Kirsten, W.J. and Hesselius, G.U. (1983) Rapid, automatic high capacity Dumas determination of nitrogen. *Microchem. J.* 28: 529-547.

Letchworth, M.B. and R.J. Lambert (1998) Pollen parent effects on oil, protein, and starch concentration in maize kernels. *Crop Science*. 38(2):363-367.

Lucas, C.J., H. Zhao, M. Schneerman S.P. Moose (2013) Changes in response to 110 cycles of selection for seed protein and oil concentration in maize. *In* Philip W. Becraft, ed, *Seed Genomics*. Wiley-Blackwell.

Maddaloni, M., G. Donini, C. Balconi, E. Rizzi, P. Gallusci, F. Forlani, S. Lohmer, R. Thompson, F. Salamini, and M. Motto (1996) The transcriptional activator Opaque-2 controls the expression of a cytosolic form of pyruvate orthophosphate dikinase-1 in maize endosperms. *Mol. Gen. Genet*. 250: 647-654.

Marks, D., Lindell, J., Larkins, B. (1995) Quantitative analysis of the accumulation of zein mRNA during maize endosperm development. *Journal of Biological Chemistry.* 260 (30): 16445-16450.

Marks, D., Lindell, J., Larkins, B. (1995) Nucleotide sequence analysis of zein mRNAs from maize endosperm. *Journal of Biological Chemistry.* 260 (30): 16451-16459.

Marzabal, P., E. Gas, P. Fontanet, J. Vicente-Carbajosa, M. Torrent & M. D. Ludevid (2008) The maize Dof protein PBF activates transcription of gamma-zein during maize seed development. *Plant Molecular Biology,* 67**,** 441-454.

Miclaus, M., J. H. Xu & J. Messing (2011) Differential Gene Expression and Epiregulation of Alpha Zein Gene Copies in Maize Haplotypes. *Plos Genetics,* 7.

Mohanty, A., Luo, A., DeBlasio, S., Ling, X., Yang, Y. Tuthill, D.E., Williams, K.E., Hill, D., Zadrozny, T., Chan, A., Sylvester, A.W., and Jackson, D. (2009) Advancing cell biology and functional genomics in maize using fluorescent protein-tagged lines. *Plant Physiology*. 149: 601-605.

Moose, S. P., J. W. Dudley & T. R. Rocheford (2004) Maize selection passes the century mark: a unique resource for 21st century genomics. *Trends in Plant Science,* 9**,** 358-364.

Prasanna, B. M., S. K. Vasal, B. Kassahun & N. N. Singh (2001) Quality protein maize. *Current Science,* 81**,** 1308-1319.

Reggiani, R., I. Brambilla & A. Bertani (1985) Effect of Exogenous Nitrate on Anaerobic Metabolism in Excised Rice Roots .1. Nitrate Reduction and Pyridine-Nucleotide Pools. *Journal of Experimental Botany,* 36**,** 1193-1199.

Schmidt, R., Ketudat, M., Aukerman, M., Hoschek, G. (1992) Opauque-2 is a transcriptional activator that recognizes a specific target site in 22-kD zein genes. *The Plant Cell.* 4: 689-700.

Sokal, R.R. and C.D. Michener (1958) A Statistical Method for Evaluating Systematic Relationships. *University of Kansas Science Bulletin*, 38, 1409 -1438.

Song, R. T., V. Llaca, E. Linton & J. Messing (2001) Sequence, regulation, and evolution of the maize 22-kD alpha zein in gene family. *Genome Research,* 11**,** 1817-1825.

Song, R. T. & J. Messing (2002) Contiguous genomic DNA sequence comprising the 19-kD zein gene family from maize. *Plant Physiology,* 130**,** 1626-1635.

Song, R. T. & J. Messing (2003) Gene expression of a gene family in maize based on noncollinear haplotypes. *Proceedings of the National Academy of Sciences of the United States of America,* 100**,** 9055-9060.

Thompson, G. A. & B. A. Larkins (1989) Structural Elements Regulating Zein Gene-Expression. *Bioessays,* 10**,** 108-113.

Tsai, C. L., I. Dweikat & C. Y. Tsai (1990) Effects of Source Supply and Sink Demand on the Carbon and Nitrogen Ratio in Maize Kernels. *Maydica,* 35**,** 391-397.

Tsai, C. L. & C. Y. Tsai (1990) Endosperm Modified by Cross-Pollinating Maize to Induce Changes in Dry-Matter and Nitrogen Accumulation. *Crop Science,* 30**,** 804-808.

Vicente Carbajosa, J., Moose, S.P., Parsons, R.L. and Schmidt, R.J. (1997) A maize zinc-finger protein binds the prolamin box in zein gene promoters and interacts with the basic leucine zipper transcriptional activator Opaque2. *Proceedings of the National Academy of Sciences of the United States of America*. 94: 7685-7690.

Viotti, A., Sala, E., Marotta, R., Alberi, P., Balducci, C. and Soave, C. (1979) Genes and mRNAs coding for zein polypeptides in *Zea mays*. *Eur. J. Biochem.* 102: 211-222.

Woo, Y.M., Hu, D.W.N., Larkins, B.A. and Jung, R. (2001) Genomics analysis of genes expressed in maize endosperm identifies novel seed proteins and clarifies patterns of zein gene expression. *Plant Cell.* 13: 2297-2317.

Xu, J. H. & J. Messing (2008) Organization of the prolamin gene family provides insight into the evolution of the maize genome and gene duplications in grass species. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 14330-5.

Xu, J. H. & J. Messing (2009) Amplification of prolamin storage protein genes in different subfamilies of the Poaceae. *Theoretical and Applied Genetics,* 119**,** 1397-1412.

CHAPTER 2. INVESTIGATION OF THE MATERNAL REGULATION OF α-ZEIN GENE EXPRESSION IN THE ILLINOIS PROTEIN STRAINS

Introduction

Three proposed mechanisms for the strong maternal effects on grain protein concentration have been proposed (Moose et al. 2004; Balconi et al. 1993). The first mechanism is endosperm dosage effect. Dosage effect arises from the double fertilization of the endosperm, where one male gametophyte fertilizes the egg cell to form a diploid embryo and the other male gametophyte fuses with the two haploid polar nuclei to form a triploid endosperm consisting of two maternal and one paternal genome (2m:1p). Effects of endosperm dosage on total and zein protein concentration have been investigated by Reggiani et al. (1985), who made reciprocal crosses between IHP and ILP and then measured protein, zein, and starch concentration. Kernels with either two (IHP x ILP) or three (IHP x IHP) doses of the IHP genome contained similar concentrations of total (approximately 21%) and zein (approximately 15%) protein. Similarly, kernels with either two (ILP x IHP) or three (ILP x ILP) doses of the ILP genome contained approximately equal concentrations of total (4-5%) and zein (1-2%) proteins. Starch concentration also exhibited similar patterns of maternal inheritance in this study. The results of this study indicate that protein concentration in the endosperm is not strictly dependent on the endosperm genotype (ie. the dosage), but rather the genotype of the maternal parent.

The second possible mechanism is genomic imprinting, a type of epigenetic modification that causes differential mRNA expression of a gene depending on the sex of the parent that transmits it. One of the most common epigenetic modifications in

plants is DNA methylation of cytosine residues by cytosine methyltransferases, which can physically block target DNA sequences from transcription factors, thereby repressing transcription. Alternatively, deacetylation and methylation of H3-K9 residues can modify chromatin structure, and the altered chromatin state can then trigger additional cytosine methylation by recruiting DNA methyltransferases (Wrage, 2005). Two types of imprinting exist: allele imprinting, where only alleles from a certain genetic background are affected by parent-of-origin-specific gene expression, and locus imprinting, where all known alleles from different backgrounds are under parent-of-origin control (Gehring et all, 2004).

Only known to occur in the endosperm tissue in plants (Gehring et al., 2004), it is hypothesized that the evolutionary role of maternal imprinting in the maize endosperm might be to maintain control of gene expression affecting kernel growth and development because of the dependency of kernel development on the maternal organ, the cob (Alleman and Doctor, 2000). Endosperm-specific expression of the α-zeins in maize is thought to result from reduced methylation in the seeds compared to leaves (Lund et al., 1995). Methylation has also been shown to contribute to allele imprinting of the α-zein genes, where heavily methylated paternal alleles repress mRNA expression compared to demethylated maternal alleles, whose expression is thus de-repressed (Lund et al., 1995; Lauria et al., 2004; for review see Bird and Wolffe, 1999). Based on these findings, Wrage (2005) hypothesized that the methylation state of the α-zein genes could affect their expression and subsequent protein accumulation in the inbred Illinois Protein Strains. Using a semi-quantitative PCR assay following restriction enzyme digestion, Wrage found differences in methylation state between leaf and seed

tissue, but not between IHP1 and ILP1. However, some unexpected results were observed and were only semi-quantitative. For example, increased cleavage of ILP1 compared to IHP1 DNA was reported by a methylation-sensitive enzyme, while it was predicted that if ILP1 was more methylated, it would be cleaved less.

The role of maternal imprinting of zein genes is investigated further here using a more quantitative assay, real-time PCR, following restriction enzyme digestion. Because zein gene expression in maize seems to be coordinately regulated, an efficient mechanism for gene silencing would be epigenetic changes to zein promoters in the regions where O2 and PBF bind. Because the O2 binding site is only 20 nucleotides downstream from the P-box, it is possible to assay methylation status of both sequences simultaneously by designing primers that flank both sequences. One method for assaying methylation is bisulfite sequencing. However, the results of a sequence-based approach may be confounded by copy number and expression variation of zein genes both within and between maize genotypes. Therefore, a qPCR based approach following restriction enzyme digestion of gDNA was employed. Differential methylation of two zein genes is assayed here in 16 days after pollination (DAP) kernels in the IHP1 and ILP1 backgrounds. It is hypothesized that if the α-zeins are transcriptionally repressed by methylation that IHP will exhibit less methylation than ILP, which would presumably result in de-repression of α-zein gene expression. Conversely, ILP would be expected to exhibit more methylation and thus repression of expression.

The third possible mechanism for the maternal effect on grain protein is nutrient supply from the source tissues. The role of nutrient source has been investigated in

IHP and ILP, beginning as early as generation 40 of the Illinois selection experiment. IHP was found to absorb more whole-plant N than ILP (Hoener and DeTurk, 1938), while ILP accumulated more carbohydrates. These observations were later attributed to elevated N uptake, N assimilation and N remobilization from vegetative tissues (source) to the grain (sink) of IHP compared to ILP, and elevated C assimilation and remobilization of ILP (Lorenzoni et al., 1978; Reggiani and Soave, 1984; Tsai et al., 1990; Dembinski et al., 1991). Subsequent amino acid profiling also demonstrated hyperaccumulation of Asn in IHP tissues (Lohaus et al., 1998). The composition of nutrients supplied by the maternal plant may represent one way storage protein synthesis is regulated by the plant, and it has been hypothesized that specifically the ratio of C to N may serve as the signal of available N assimilates for protein synthesis. The large differences in C and N metabolism and protein concentration between IHP and ILP are consistent with this hypothesis.

The high number and high sequence homology of α-zein genes, the presence of pseudogenes, and the quantitative inheritance of grain protein complicate approaches for studying individual zein gene expression. The use of fluorescent-protein promoter reporter lines provide an alternative method for tracking α-zein expression. Derived from the coral reef species, *Discosoma,* a monomeric version of the tetrameric DsRed protein termed mRFP1 (Campbell et al. 2002) was fused to the C-terminus of the Z22α-FL2 zein gene, *Floury2. Floury2* has been shown to account for approximately 20% of all Z22α expression in inbred variety B73 with only one gene expressed more (Song and Messing 2003, Feng et al. 2009) and also highly expressed in the IPS (Lucas et al., 2013). To preserve proper tissue and temporal regulation of the *Floury2*-mRFP1 fusion

protein, the construct includes an intact α-zein gene driven by native flanking regulatory elements, including approximately 2000-bp of the *Floury2* gene promoter and 1000-bp of 3' sequence. When expressed in the endosperm, the DsRed protein can be visualized under standard white light, with the kernels appearing pink-to-red in color (Wenck et al. 2003). This property can be attributed to the high level of *Floury2* gene expression, the relatively high stability of zein proteins, and the use of the monomeric RFP that does not require multimerization to emit fluorescence (Campbell et al. 2002). As a result, *Floury2* gene expression can be rapidly monitored during seed development and quantified by direct imaging, and does not require sample destruction. While the experiments in this chapter rely only on qualitative or only semi-quantitative observations of *Floury2*-mRFP1 expression, a precise method for quantifying *Floury2*-mRFP1 is described in chapter three. Inclusion of the native regulatory sequences may allow these lines to provide more information about regulatory elements acting at the level of transcription.

The *Floury2*-mRFP1 construct was originally transformed into maize genotype Hi-II using the *Agrobacterium* method. The Hi-II transgenic plants were then crossed to inbred variety B73, and the resulting F1 transgenic seeds were supplied by Dr. Dave Jackson from Cold Spring Harbor. Approximately 20 seeds were obtained from three transgenic events, named 47, 52 and 172. Introgression of the mRFP1 transgene into the inbred IPS was begun by Salas (2008) by crossing the F1 (B73 x Hi-II) *Floury2*-mRFP1 reporter lines to inbreds IHP1, ILP1, IRHP1, IRLP1 and B73. The hybrids generated from these crosses were then backcrossed to the inbred IPS (IHP1, ILP1, IRHP1 and IRLP1) and B73, and BC1 seed was available at the start of this project.

Initial analysis of the *Floury2*-mRFP1 reporter lines by Wenck et al. (2003) revealed the ability to visualize transgene expression under white light in a quick and nondestructive manner, the kernels appearing pink to red in coloration. This type of visual phenotyping was used to evaluate the developmental and spatial expression of the transgene by Lucas et al. (2013) when backcrossed to the inbred IPS and inbred variety B73. As determined by the pink-to-red endosperm color, *Floury2*-mRFP1 expression began around 10 DAP and increased throughout grain fill, consistent with endogenous zein expression. Additionally, *Floury2*-mRFP expression patterns were found to closely follow overall 22-kD α-zein expression in IHP1 and ILP1, with a 3- to 4-fold higher expression in IHP1 (Lucas et al., 2013). Overall, these results demonstrated proper regulation of the *Floury2*-mRFP1 transgene regardless of genomic location and the ability for the *Floury2*-mRFP1 pink-to-red kernel color phenotype to report on zein gene expression. For this reason, the *Floury2*-mRFP1 phenotype was employed here in a series of crosses to investigate the maternal regulation of grain protein concentration. The effects of dosage, genomic imprinting and plant nutrient status on *Floury2*-mRFP1 expression in the inbred IPS and B73 backgrounds are presented here per **objective 1**.

Materials and Methods

Plant Material

For the methylation assay, inbreds IHP1 and ILP1 plants were grown under field conditions at the Department of Crop Sciences research and education center in Champaign, Illinois during the 2008 growing season. 15 seeds were planted in 12 foot plots in 30 inch rows under 0 applied N if planted in the summer nursery. Plants were selfed, and 16 days after pollination (DAP) kernels were collected and flash frozen. With respect to the series of reciprocal crosses, introgression of the mRFP1 transgene into the inbred-derived Illinois Protein Strains (IPS) was begun by Salas (2008) by crossing the mRFP1 reporter lines to the inbred lines IHP1, ILP1, IRHP1, IRLP1 and B73. The hybrids generated from these crosses were backcrossed to inbred IPS and B73, and BC1 seed was available at the start of this project. Seeds were grown in either field or greenhouse conditions at the Department of Crop Sciences research and education center in Champaign, Illinois during the 2008–2013 growing seasons. 15 seeds were planted in 12 foot plots in 30 inch rows under 0 applied N if planted in the summer nursery. If grown in the greenhouse, four seeds were planted per 5L pot and the plants thinned to one plant per pot. Kernels planted in the greenhouse were grown to maturity under a daily light/temperature regime of 16 hours at 28 degrees C and eight hours at 22 degrees C and irrigated daily by drip irrigation.

Because the presence of the transgene can be identified easily and non-destructively by the pink coloration of the kernels, only pink BC1 kernels were planted for further backcrossing. The inbred IPS and B73 were planted at a 10-day delay to account for hybrid vigor of the BC1s. The inbred lines were used as the female in

backcrosses to maximize genomic recovery of the recurrent parent. Subsequent backcrosses were created by alternating plantings in the greenhouse and summer nurseries. To ensure stable behavior of at least one of the three reporter lines, all three of the transgenic events (47, 52 and 172) were backcrossed to all strains until BC4. Analysis of the BC4 IPS:*Floury2-mRFP1* seed revealed no differences in *Floury2-mRFP1* expression patterns due to transgenic event, so only event 52 was backcrossed further to the IPS, and is the only event used in the experiments documented here. All three transgenic events were fully backcrossed to B73. In addition to backcrossing the *Floury2*-mRFP1 transgene, reciprocal crosses were made between the IPS and B73 to test for the source of the maternal effect on *Floury2*-mRFP1 expression where the transgene was transmitted through both maternal and paternal genotypes.

Ears resulting from either backcrossing or reciprocal crosses were photographed using a Nikon D60 digital camera. Backcrossed ears had undergone a minimum of six generations of backcrossing following a varied number of selfing generations, as follows: IHP1 ears were BC7S1; ILP1 and IRHP1 ears BC7S4; IRLP1 ears BC6; and B73 ears BC6S1. Reciprocal crosses were also made between the IPS and B73 plants.

Genomic DNA Extraction

DNA extraction was conducted using TRIzol ® LS Reagent following the Life Technologies manufacturer protocol (https://www.lifetechnologies.com/order/catalog/product/10296028).

Primer Development

Optimal primers which specifically amplified the targeted DNA sequence of the gene of interest were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi). BLAST searches confirmed the total gene specificity of the primer sequences. Due to the high copy number and high sequence homology of zein genes, it is notoriously difficult to design primers that uniquely amplify a single zein gene sequence. An attempt was made to design unique primers to zein genes that have been shown to be expressed in the inbred IPS in prior EST sequencing studies conducted by Moose lab members (data unpublished). The following 22kD α-zein genes were assayed: 22-4, 22-11, z1C1-1, z1C1-2, z1C1-3, z1C1-5, z1C1-6, z1C1-7, and z1C2-1 (*Floury2*). The following 19kD α-zein genes were assayed: z1A2-1 and z1B1-4. However, it was only possible to design primers for z1B1-4 (19kD) (gi240252476) and z1C2-1 (*Floury2;* 22kD*)* (AF090447.2). The respective amplified products are 262 and 202 basepairs. It was also desirable to include a positive control, and a member of the *Opie2* family of retrotransposable elements was chosen for this purpose because it is located within the clusters of tandemly-repeated zeins and is known to be methylated (GenBank accession number U68408). The *Opie2* primers amplify a pol/gag region. The sequences below are oriented in the 5' to 3' direction.

Z1B1-4 Forward: TAACATTGGTGTTACTGCCATAAAATA; Reverse: TAGACCTTGTGCTTATGTAGATTGAGTT

Z1C2-1 Forward: TGGCACTTACTCGATTTTGACA; Reverse: AACCAGCAGAACAATACTACAACAA

Opie2 Forward: AGAGGAAGGGATCAAGCACG; Reverse:

TAGACCCTGTTGATGGCGTGG

PCR Amplification of DNA

In order to test for unique amplification of gene products, the above primer sets were used in PCR of DNA extracted from 16 DAP samples from inbreds IHP1 and ILP1. 10x Standard Taq Reaction Buffer (NE Biolabs), dNTPs (Biorad), and Taq polymerase (NE Biolabs) were used for the PCR following manufacturer protocol. The PCR amplification profile used an initial denaturation step of 95°C for 2min followed by 34 cycles of 95°C for 30s, 52°C for 1min, and extension at 72°C for 1min. Additionally, primer efficiency was tested by qPCR on a dilution series of five DNA concentrations (5, 10, 20, 40 and 80ng).

Methylation Assay

The methylation assay used here is described in Bedell et al. (2005) and is a real-time PCR technique that reports DNA methylation status of genomic DNA. The assay compares cycle thresholds (C_Ts) of gDNA that have been subjected to enzymatic treatments, where the C_T value of a locus is the function of the number of copies present within the assay tube or plate. The assay is based on the formula that the total number of gene copies = the number of methylated copies + the number of unmethylated copies. Typical sample assays include four treatments, including: 1.) a mock-digested treatment, which reports the total number of copies present, 2.) the methylation-sensitive treatment, where an equivalent amount of gDNA is treated with a

methylation-sensitive restriction enzyme (MSRE) and which reports on the number of gene copies that are methylated, 3.) the methylation-dependent treatment, where an equivalent amount of gDNA is treated with a methylation-dependent restriction enzyme (MDRE) and which reports on the number of gene copies that are unmethylated, and D.) the double digestion with both the MSRE and MDRE. Here, a fifth treatment was also included using a restriction enzyme that recognizes the same sequence as the MSRE, but is less sensitive to DNA methylation. For methylated loci, the C_T from the MSRE treatment should be the same as the untreated control, and the C_T from the MDRE greater. For unmethylated loci, the C_T from the MDRE should be equal to that of the untreated control and the C_T from the MSRE greater.

One *Sau3A*I / *Mbo*I (MSRE) cleavage site was identified within the 202 bp *Z1C2-1 Floury2* amplification product, and two *Sau3A*I / *Mbo*I (MSRE) cleavage sites were identified within the 262 bp *Z1B1-4* product (Figure 2.1). Cleavage with *Sau3A*I is blocked when the substrate DNA is CpG methylated. *Mbo*I recognizes the same site as *Sau3A*I; however, it is only impaired with overlapping methylation. *MspJ*I (MDRE) detects fully methylated CpG or CHG sites and do not cleave unmodified DNA; numerous *MspJ*I sites are predicted. Restriction enzyme cocktails were prepared according to New England Biolabs manufacturer protocols with the exception that volumes were decreased by half.

DNA was diluted to 100ng/uL for all genotypes. A 275μL MSRE (*Sau3A*I) reaction cocktail was prepared using conditions specified by NEB, with the exception that volumes were reduced by half. *Sau3A*I enzyme was initially withheld from the cocktail in order to aliquot appropriate volumes into the subsequent treatments.

Approximately 3300ng (33μL) gDNA was added to the cocktail for a total volume of (275-22+33) 286μL, and mixed by pipetting. 120μL, 120μL and 60μL of this master mix were then aliquotted into three separate 1.7μL tubes labeled B, C and D. Eight μL of dH_20 was added to B as a mock treatment, 8μL MSRE (*Sau3A*I) was added to C, and 4μL dH_20 was added to D. A 60μL (4 Units) MSRE (*Mbo*I) reaction cocktail, including 4μL *Mbo*I, was prepared, and the mix was added to D for a total volume of 124μL. Tubes B, C and D were digested overnight at 37°C. A 250μL MDRE (*MspJ*I) reaction cocktail was prepared using conditions specified by NEB, with the exception that volumes were reduced by half. *MspJ*I enzyme was initially withheld from the cocktail in order to aliquot appropriate volumes into the subsequent treatments. 54μL of the mix was aliquotted into four separate 1.7μL tubes labeled J, K, L and M. 4μL of dH_20 was added to each J and M instead of enzyme, and 4μL MDRE (*MspJ*I) was added to each K and L. Mixes were vortexed for 10 secs and incubated for 4hr at 37°C. The samples were then aliquotted into a 96-well plate. 18μL of J was added to each of 3 columns of row E, 18μL of K to F, 18μL of L to G, and 18μL of M to H. Then 20μL of the digestions from the previous day were aliquotted to the same wells. 20μL of B to E and F, 20μL of C to G and H, and 20μL of D to D. In this way, three digestion replications were conducted for five treatments: row D contained the MSRE (*Mbo*I) treatment; E the mock treatment; F the MDRE (*MspJ*I) treatment; G the double digestion (both *Sau3A*I and *MspJ*I); and H, the MSRE (*Sau3A*I) treatment. The plate was digested overnight at 37°C. The plate was heated for 10min at 95°C to deactivate the enzymes, followed by qPCR of gDNA.

Quantitative Real Time PCR

Quantitative real time PCR on inbreds IHP1 and ILP1 was performed for 3 digestion replications. SYBR Green PCR Master Mix (Applied Biosystems Inc., Foster City, CA) was used in RT-PCR for relative quantitation of genomic DNA following enzymatic digestions, following the manufacturer's protocol. Amplification and detection were performed on a DNA Engine Opticon 2 (Bio-Rad Laboratories, Hercules, CA). Melt curve analysis was conducted following each reaction to confirm the presence of only a single product of the reaction. Relative DNA abundances were calculated by normalizing all treatments for a given gene to the ILP1 mock treatment for that gene using the following equation: $[(\text{Primer Eff})(2)^{\wedge CT(IHP1:rep1)} + (\text{Primer Eff})(2)^{\wedge CT(IHP1:rep2)} + (\text{Primer Eff})(2)^{\wedge CT(IHP1:rep3)} / 3] / [(\text{Primer Eff})(2)^{\wedge CT(ILP1:rep1)} + (\text{Primer Eff})(2)^{\wedge CT(ILP1:rep2)} + (\text{Primer Eff})(2)^{\wedge CT(ILP1:rep3)} /3]$ where Primer Eff = primer efficiency and CT(IHP:rep1) = cycle threshold of IHP1 genotype, replication 1, etc. According to the mock treatments, ILP gDNA input was greater than IHP1 input. Therefore, it was necessary to standardize relative IHP1 gDNA abundance due to mock treatment by multiplying calculated values from above by 20.37 for *Floury2* treatments, 4.34 for *z1B1-4* treatments or 10.64 for *opie2* treatments. Relative gDNA abundances following each treatment are reported.

Results

Methylation Assay to Test for Maternal Imprinting of Zein Genes

To test whether differential methylation of α-zein loci might contribute to the differences in α-zein gene expression (Lucas et al., 2013) and protein abundance

(Bhattramakki et al., 1996) between IHP1 and ILP1, a methylation assay was conducted that employs real-time PCR to report DNA methylation status of genomic DNA (Bedell et al., 2005). It was only possible to develop primers unique to two zein genes, the *Z1B1-4* 19kD and the *Z1C2-1* (*Floury2)* 22kD α-zein genes. The primers were designed to flank the P-box and the O2 binding sites in *Z1C2-1* and the P-box in *Z1B1-4* (19-kD α-zeins do not contain an O2 binding site), which are located approximately 300bp upstream of the transcriptional start of zein genes (Vicenta-Carbajosa et al., 1997). One *Sau3AI/ MboI* (MSRE) recognition site was identified within the targeted promoter sequence in *Z1C2-1*, and two were identified in *Z1B1-4*, according to B73 reference sequence. The primer binding sites and *Sau3AI* (MSRE) restriction enzyme sites are depicted in the gene diagrams of *Z1B1-4* and *Z1C2-1* (*Floury2)* in Figure 2.1. *Opie2* primers were included as a positive, methylated control.

To test for unique primer amplification products, gDNA from IHP1 and ILP1 16 DAP kernels was PCR amplified and the products run for 2 hours on an agarose gel for size separation. The results indicated only a single band product for all three genes and both genotypes (data not shown). Additionally, the bands were the appropriate sizes; *Z1B1-4* primers produced a 262bp product, *Z1C2-1* primers produced a 202bp product, and opie2 primers produced a 170bp product. Opie2 primers produced significantly brighter bands than either zein gene. To test primer efficiency at various DNA levels, qPCR was conducted on a dilution series of a single gDNA sample, IHP1 (16 DAP kernel). This assay included five gDNA concentrations, including 5, 10, 20, 40 and 80 ng of gDNA. gDNA concentration was regressed with observed C_T values, and R^2 values were calculated for each primer. R^2 values were 0.88, 0.91, and 0.90 for the

Z1B1-4, Z1C2-1 and *Opie2* primers, respectively. Additionally, *Opie2* C_T values were much lower than C_T values for either zein gene, indicating a greater abundance of gDNA.

It was hypothesized that hypermethylation of ILP1 zeins might contribute to lower protein abundance by gene silencing. The methylation assay included three digestion replications for each of five treatments for two genotypes and three genes. Treatments are described in detail in the materials and methods section. Cycle threshold (C_T) values were averaged for the three digestion replications. Due to observed differences in DNA input between IHP1 and ILP1, where the amount of ILP1 DNA was always greater (lower C_T values), C_T values were normalized to ILP1. Fold-reduction in DNA due to treatment is plotted on a $\log_2$ scale in Figure 2.2. The basis for interpreting methylation assay results is that *greater* $\log_2(C_T)$ values indicate *less* gDNA amplification by qPCR, and less gDNA indicates greater cleavage by the restriction enzymes. For *completely methylated* loci, the C_T from the MSRE treatment should be the same as the untreated control (mock treatment), and the C_T from the MDRE greater. For *unmethylated* loci, the C_T from the MDRE should be equal to that of the untreated control (mock treatment) and the C_T from the MSRE greater.

For *Opie2*, $\log_2(C_T)$ values for MDRE and double digestions were always greater than any other treatment for both genotypes, indicating that *Opie2* loci are methylated, as expected. However, the slightly greater $\log_2(C_T)$ values for the MSRE treatment compared to the mock, especially for ILP1, indicate that the loci are not completely methylated because there is some cleavage. Overall, these results demonstrate greater methylation of *Opie2* than the zeins.

For *Z1B1-4* and *Z1C2-1 (Floury2)*, the $\log_2(C_T)$ values for MDRE and double digestions are always greater than any other treatment for both genotypes, suggesting that these sites are methylated and thus cleaved. For ILP1, there was no amplification of DNA for MDRE or the double digestion, indicating complete cleavage. Combined with the fact that there was more ILP1 DNA to begin with, this observation strongly suggests greater methylation of ILP1 zein genes. To the contrary, IHP1 mock treatments indicated less DNA to begin with (higher $\log_2(C_T)$ values) and more DNA after MDRE treatments (lower $\log_2(C_T)$ values), indicating less cleavage and a lower degree of methylation.

If the loci were completely methylated then the MSRE (*Sau3AI*) C_Ts should have been approximately equal to the mock C_Ts because all cleavage would have been blocked. However, the MSRE $\log_2(C_T)$ values were actually greater than mock $\log_2(C_T)$ values for both genotypes, but still less than MDRE C_Ts. This means that there was some cleavage by the MSREs and that the loci are only partially methylated. While *Sau3AI* is completely blocked by methylation, *MboI* is only partially blocked by methylation. Therefore, the $\log_2(C_T)$ values for the *MboI* treatment should be slightly greater than those for *Sau3AI* if the locus is methylated because some cleavage may still occur. This seems to be true for ILP1. However, if the locus is unmethylated then *Sau3AI* and *MboI* will both cleave equally, resulting in equal $\log_2(C_T)$ values. For IHP1, $\log_2(C_T)$ values for *Sau3AI* were actually greater than those for *MboI.* This result is unexpected all together and could result from different amounts of enzyme. For example a greater amount of *Sau3AI* enzyme than *MboI* enzyme would lead to greater $\log_2(C_T)$ values. As a result, the *Sau3AI /MboI* comparison is unreliable. Overall,

greater reductions in DNA were observed in ILP1 due to MSRE treatments (*Sau3AI* or *MboI*) than in IHP1, which might suggest less methylation (and thus greater cleavage) in ILP1. This is not consistent with the results of the MDRE treatments that indicate more methylation in ILP1. The discrepancy could be due to higher amount of input DNA for ILP because it provides a greater dynamic range for which to detect differences. However, because the *MspJI* enzyme can recognize any potential methylation in the amplified region but *Sau3AI* or *MboI* only test for methylation at a few bases, more confidence should be given to the MspJI results that indicate more methylation in ILP compared to IHP.

Due to sheer differences in DNA between IHP1 and ILP1 in the digestion, the DNA was quantified by qPCR (sans digestion treatments). However, differences of only 1.4-fold (ILP1>IHP1) for *Z1C2-1* and *Opie2* and 2-fold (IHP1>ILP1) for *Z1B1-4* were observed, and only small differences were observed between three technical qPCR replications. Furthermore, no shearing was evident when the DNA was run on an agarose gel, and A_{260} values were about 1.8. Finally, as demonstrated earlier, the primers amplified even small amounts of DNA as efficiently as greater amounts of DNA.

Floury2-mRFP1 Expression when Backcrossed to the Inbred Illinois Protein Strains

The *Floury2*-mRFP1 was backcrossed a minimum of six generations, followed by a varying number of selfing generations (see Materials and Methods section for exact number), to the inbred IPS and B73. Throughout backcrossing, selection for *Floury2*-mRFP1 was simply achieved through visual selection for pink kernels. The resulting

ears were segregating for a single *Floury2*-mRFP1 (hemizygous) transgene locus, with only approximately half of the kernels transgenic, and are shown in Figure 2.3. Visual inspection of the ears indicated endosperm-specific expression, where the intensity of the pink to red kernel color phenotype was consistent with known protein concentrations of the inbred IPS and B73. IHP1 kernels contained the highest protein (27%) and were the darkest pink; ILP1 and IRHP1 kernels contain the lowest protein (5% and 7%, respectively) and were the lightest pink; IRLP1 kernels contain an intermediate amount of protein (15%) and were intermediate in color to ILP1 and IHP1 kernels. B73 kernels contain a relatively low protein abundance (8%) and were also fairly light in color.

In addition to selection for the presence of the *Floury2*-mRFP1 transgene, selection for general ear phenotypes was maintained throughout backcrossing to recover the phenotype of the wild-type inbred IPS, as opposed to either B73 or Hi-II ear phenotypes from whose genetic backgrounds these lines are derived. While B73 is characterized by yellow kernels and a red cob, the IPS are derived from a white-kerneled and white–cobbed variety, Burr's White. Analysis of Figure 2.3 illustrates that the transgenic inbred IPS contained white kernels and cobs, indicating recovery of the recurrent parent ear phenotypes. Additionally, IHP1 and IRLP1 kernels were smaller and vitreous, while ILP1 and IRHP1 kernels were larger and opaque. These observations are consistent with their wild-type phenotypes, and also indicate recovery of the recurrent parent genome.

Floury2-mRFP1 Expression in Reciprocal Crosses Between the Inbred Illinois Protein Strains and B73

After introgression to the inbred IPS and B73, a series of reciprocal crosses were made among the transgenic lines in order to test for the source of maternal effect on grain protein concentration. Four types of crosses were made, varying the maternal and paternal genotypes, as well as the parent transmitting the *Floury2*-mRFP1 transgene, and the ears are shown in Figure 2.4. The ears in the top half of the figure were generated with *Floury2*-mRFP1 transgene transmitted through the maternal genotype, and the ears in the bottom half of the figure were generated with the transgene transmitted through the male genotype. Ear genotypes are indicated either above (paternal) or below (maternal) the ears. When the IPS were crossed as males onto B73, regardless of whether *Floury2*-mRFP1 was transmitted through the female (upper right) or male (lower right), the ears appeared more B73-like in size and shape, and the kernels appeared approximately the same pink coloration. However, when the IPS were used as females and B73 crossed onto them as males, the pink coloration of the kernels corresponded to endogenous *Floury2* expression and known protein concentrations of the inbred IPS, regardless of whether *Floury2*-mRFP1 was transmitted through the female (upper left) or male (lower left).

Discussion

The α-zeins exhibit allele imprinting, where maternal alleles are de-methylated and thus de-repressed (Lund et al., 1995). Based on these results, Wrage (2005) hypothesized that differential methylation of α-zein loci might contribute to the

differences in both α-zein gene expression and protein abundances between IHP1 and ILP1. While Wrage concluded no differences in methylation between IHP1 and ILP1, further investigation was recommended due to somewhat unclear and/or unexpected results in parts of the analysis. Therefore, an objective here was to assay methylation status of two zein loci, *Z1B1-4* and *Z1C2-1* (*Floury2)*. Due to the abundance of pseudo genes and high degree of presence-absence variation of the α-zein genes in maize, it was first necessary to reference a previously identified subset of α-zein genes whose expression has been verified by EST sequencing in the inbred Illinois Protein Strains (data unpublised). Of this subset, an attempt was made to develop primers that would uniquely amplify the P-box and O2 binding sites of 11 gene copies, including nine 22kD and two 19kD α-zeins. Due to the high copy number and high sequence homology of zein genes, of the 11 promoter sequences assayed, it was only possible to develop primers unique to each of two genes, the *Z1B1-4* 19-kD and the *Z1C2-1* (*Floury2)* 22-kD α-zein genes. Unique primer amplification was confirmed by running the PCR products on an agarose gel, which demonstrated single bands of the proper size for all three primers and both genotypes. The observation of brighter bands and lower C_T values for *Opie2* products is consistent with the greater amount of *Opie2* genomic sequences compared to zein genomic sequences. Although it was possible to develop unique primers for two zeins, the large number of genes for which unique primers could not be developed attests to the difficulty associated with studying individual zein gene expression.

The results of the MDRE treatments indicated significant DNA cleavage for both IHP1 and ILP1. However, because there was more ILP1 DNA to begin with and

virtually none detected by the MDRE treatment, this suggests that the zein loci assayed here are more methylated in ILP1 compared to IHP1. While the greater cleavage of DNA in ILP1 for the MSRE treatment compared to IHP1 may suggest less methylation in ILP1, more confidence should be given to the results of the MDRE treatment because of the abilily of this enzyme to detect potentially any methylated site compared to only a few sites by the MSRE. To help clarify results, the experiment could be repeated with equal DNA input.

A few sources of variation for the observed differences in DNA quantity could be pipetting errors and variation, differences in primer efficiency depending on DNA quantity, and DNA degradation. To test for differences in DNA quantity, the samples were quantified by qPCR (sans digestion treatments), but differences of only 1.4-fold (ILP1>IHP1) for *Z1C2-1* and *Opie2* and 2-fold (IHP1>ILP1) for *Z1B1-4* were observed, and only small differences were observed between three technical qPCR replications. Original mock treatments indicated up to 32-fold differences. No shearing was evident when the DNA was run on an agarose gel, and A_{260} values were about 1.8. Finally, the primers amplified all DNA quantities efficiently. These results indicate that the source of variation is not due to pipetting, primer efficiency or DNA degradation. On the other hand, the source of variation could arise from differences in IHP1 and ILP1 digestions. However, the exact same procedure was used for each genotype, and the digestions and subsequent qPCR for each respective genotype were conducted within the same 96-well plates. It is also possible that IHP1 DNA became contaminated with a restriction enzyme.

While the results of the methylation assay did seem to suggest greater methylation of zein loci in ILP1 that could indicate imprinting, there were some discrepancies that could not be resolved. Fortunately, the results from reciprocal crosses with the *Floury2*-mRFP1 transgene did provide a more conclusive result as to the source of the maternal effect. The *Floury2*-mRFP1 transgene offers an alternative means of tracking α-zein protein in the kernel, and it has been shown to closely follow 22-kD α-zein gene expression in the inbred IPS (Lucas et al. 2013). The *Floury2*-mRFP1 transgene was backcrossed a minimum of six generations to the inbred IPS and B73. By BC6, approximately 99.2% of the recurrent genome is predicted to be recovered. The recovery of wild-type protein concentrations and general ear phenotypes, including cob color, kernel vitreousness, size and color, in the inbred IPS and B73 indicates that factors governing these traits were successfully introgressed by BC6. That the *Floury2*-mRFP1 phenotype closely followed known protein concentrations attests to complete introgression and proper regulation, which is also supported by the finding that transgene expression was greatly reduced by the *o2* recessive null mutation in the IHP1 background (Lucas et al., 2013). Continued selfing until BC7S6 is recommended to ensure complete fixation of these genotypes.

A series of crosses were made among the transgenic inbred IPS and B73 to test for possible sources of the maternal effect on grain protein concentration. The first possible mechanism by which the maternal effect can arise is dosage effect due to the triploid nature of the endosperm. It is possible for the *Floury2*-mRFP1 transgene to vary from zero to three doses, depending on whether it is inherited from the female or male parent, and if it is heterozygous or homozygous. Because *Floury2*-mRFP1 transgene

expression is visible in the kernel, kernel color could be monitored as a result of varying dosages of the transgene. When plants heterozygous for the *Floury2*-mRFP1 transgene were crossed to the inbred IPS, the ears were expected to segregate for either zero and one *Floury2*-mRFP1 allele (if inherited through the male), or zero and two alleles (if inherited through the female) for a net difference of one copy of the transgene. Ears containing two copies of the transgene would be predicted to exhibit pink coloration up to twice the strength of ears containing only one copy of the transgene if dosage effect was important. However, no difference in kernel coloration was observed due to dosage, indicating that endosperm dosage is not the mechanism for the observed maternal effect. The series of reciprocal crosses by Reggiani et al. (1985) between IHP and ILP are consistent with this finding.

To investigate the role of genomic imprinting, *Floury2*-mRFP1 expression was monitored for parent-of-origin differences. In this experiment, progeny seed color is contingent upon direction of the reciprocal cross *only if the transgene is imprinted*. For example, if the transgene was maternally imprinted then progeny kernels would appear pink in color only if transmitted by the female. Conversely, if the transgene was paternally imprinted then progeny kernels would appear pink in color only if transmitted by the male. However, the results from reciprocal crosses between the transgenic inbred IPS and B73 illustrate the presence of transgene expression regardless of which parent transmitted it. These observations demonstrate that neither maternal nor paternal imprinting alter *Floury2*-mRFP1 transgene expression. However, because imprinting often effects gene clusters, and it is unlikely that the *Floury2*-mRFP1 transgene was incorporated into one of the five zein clusters on chromosomes one, four

and seven, imprinting cannot be ruled out. Sequencing transgenic lines would allow mapping the precise location of the transgen in the genome.

The lack of support for either endosperm dosage or maternal imprinting leaves nutrient supply from the source tissues as the most likely mechanism for maternal control of zein gene expression and seed protein concentration. The observation of invariable kernel color within segregating ears suggests that regulation of *Floury2*-mRFP1 transgene expression does not occur on an individual kernel basis, but rather at the level of the entire ear. This implies that zein synthesis within the endosperm is a downstream process and subject to regulatory factors acting upstream. The seed represents a strong nitrogen sink and is reliant upon the plant for delivery of N-assimilates. Therefore, it is feasible that quantity or composition of nutrients within the plant may serve as a signal of the vegetative nutrient status of the plant that is available for grain fill. In support of this, several studies have demonstrated a response of protein to N supply. For example, zein accumulation was shown to increase proportionately to N supply *in vitro* within the W64A background (Singletary et al., 1990). Uribellarrea et al. (2004 and 2007) also found that when the inbred-derived Illinois Protein Strains were crossed to an elite common tester *in vivo*, the hybrids exhibited a more than two-fold difference in grain protein. This suggests that the large differences in N and C uptake, assimilation and remobilization between IHP and ILP could be important determinants of kernel composition. The genetic effects of factors thought to affect whole-plant N and C metabolism, namely enzymes in the asparagine (Asn)-cycling pathway, are investigated further in chapter 3.

Conclusion

The maternal effect on grain protein concentration is widely documented in a variety of cereals and legumes, including IHP and ILP maize varieties. Three mechanisms of maternal inheritance were tested here by a combination of tests for epigenetic modification and through the monitoring of *Floury2*-mRFP1 expression in a series of reciprocal crosses between the inbred IPS and B73. While transgenic experiments can only provide information about *Floury2* regulation, it is hypothesized that all α-zein genes would be subject to the same regulatory mechanisms in accordance with their coordinated regulation (see chapter three). The results of the methylation assay did suggest greater methylation of ILP1 zeins compared to IHP1 zeins, which could, but not necessarily, indicate maternal imprinting. Based on these findings, it is possible that differential methylation of zein loci between IHP1 and ILP1 may contribute to respective differences in gene expression and protein abundance.

The expression of *Floury2*-mRFP1 regardless of which parent transmitted it strongly suggested a lack of imprinting. However, the transgene likely incorporated into a genomic location other than the zein clusters, and because gene clusters are more likely to be imprinted, it is still possible that the zeins are imprinted even though *Floury2*-mRFP1 is not. Pinpointing the genomic location of the transgene will be necessary to confirm that it did not incorporate into a zein cluster. Equivalent expression of *Floury2*-mRFP1 regardless of dosage strongly suggested that transgene expression is not governed by dosage effect, which is consistent with prior studies.

Two observations from the *Floury2*-mRFP1 expression analyses strongly supported the role of plant nutrient status. First, no differences in kernel color were

observed within segregating ears. The uniformity of kernel color suggests that the mechanism governing grain protein concentration acts on the level of the entire ear, rather than on an individual kernel basis, and could be governed by the plant itself (eg. plant nutrient status). Secondly, differences in kernel color were consistent with grain protein concentration only when the female genotype was varied. It is hypothesized that the nutrient status of the maternal plant is a mechanism by which the plant retains control over the quantity and composition of nutrients entering the ear for grain fill. Enzymes in the Asn-cycling pathway influence the composition of nutrients in plant tissues, and the loci encoding these enzymes represent strong gene candidates. The impact of genetic variation for the genes encoding Asn-cycling enzymes is investigated in chapter three as a means of further testing the hypothesis that plant nutrient status largely dictates kernel nitrogen accumulation.

Figures

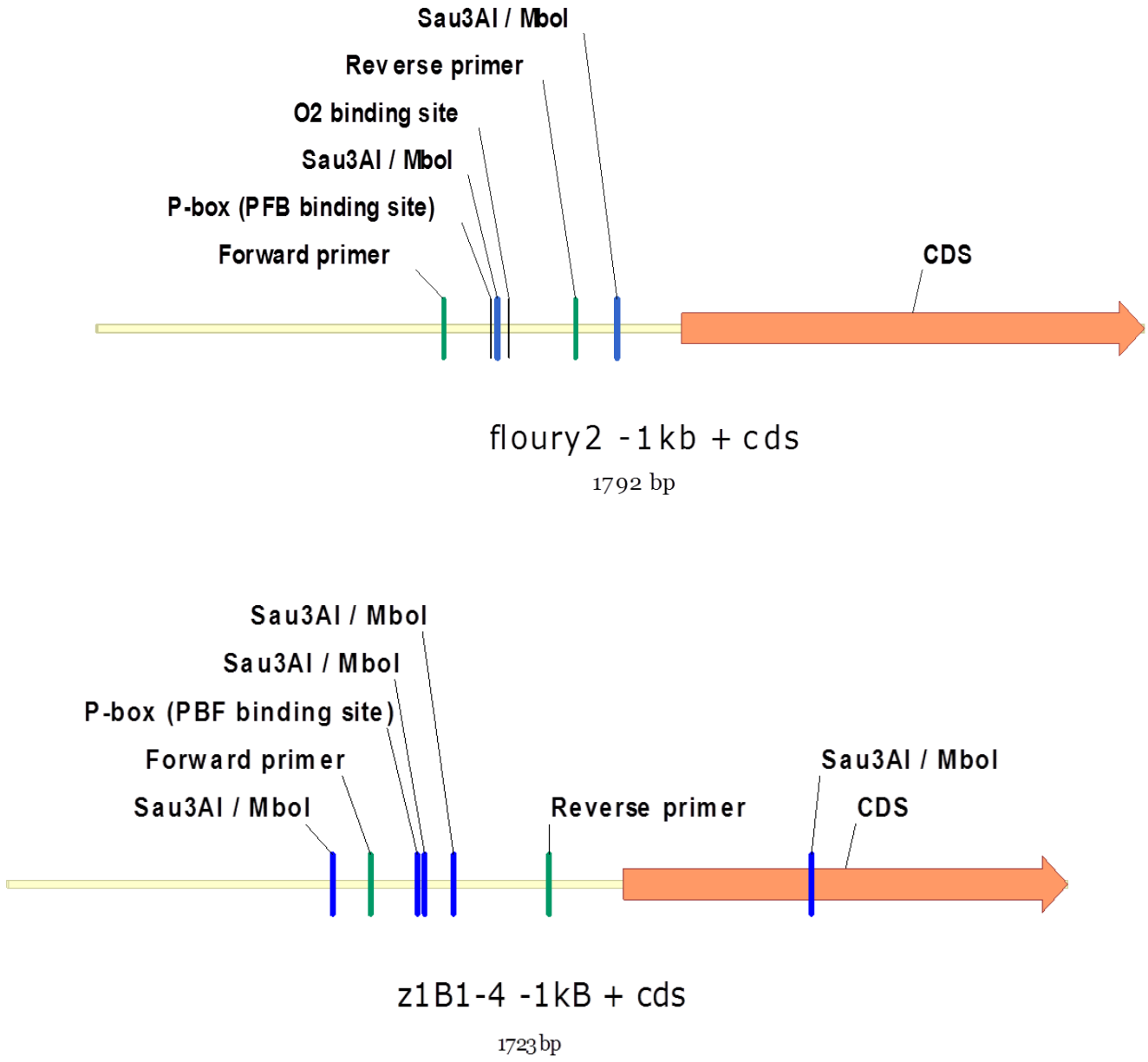


Figure 2.1. *Z1C2-1* (*Floury2*) and *Z1B1-4* gene diagrams showing primer sites and restriction enzyme recognition sites. Coding sequences, primer binding sites, and the P-box and O2 binding sites are indicated.

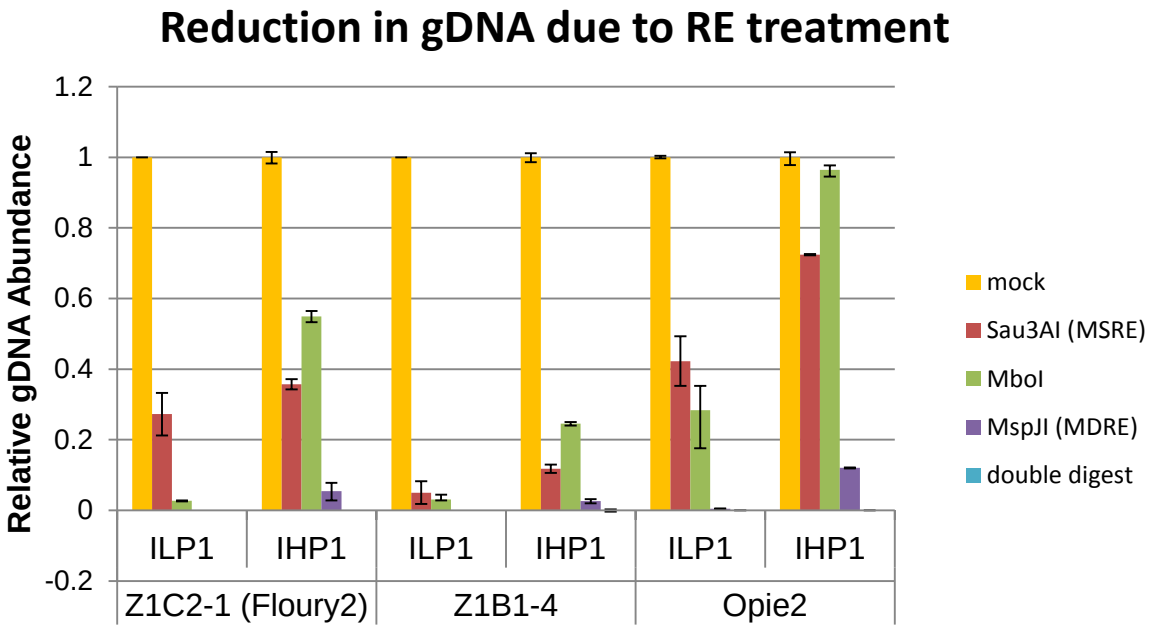


Figure 2.2. Relative gDNA abundance following restriction enzyme digestion treatments for *Z1B1-4* 19kD and *Z1C2-1* 22kD α-zein gene promoters in Illinois High Protein (IHP1) and Illinois Low Protein (ILP1) inbred 16 days after pollination (DAP) kernels. *Opie2* was included as a positive, methylated control. MSRE indicates methylation-sensitive restriction enzyme (*Sau3AI*), which does not cleave if methylated. MDRE indicates methylation-dependent estriction enzyme (*MspJI*), which only cleaves if methylated. *MboI* recognizes the same sequence as *Sau3AI*, but cleavage is only partially blocked by methylation. The double digestion contains both MSRE and MDRE.

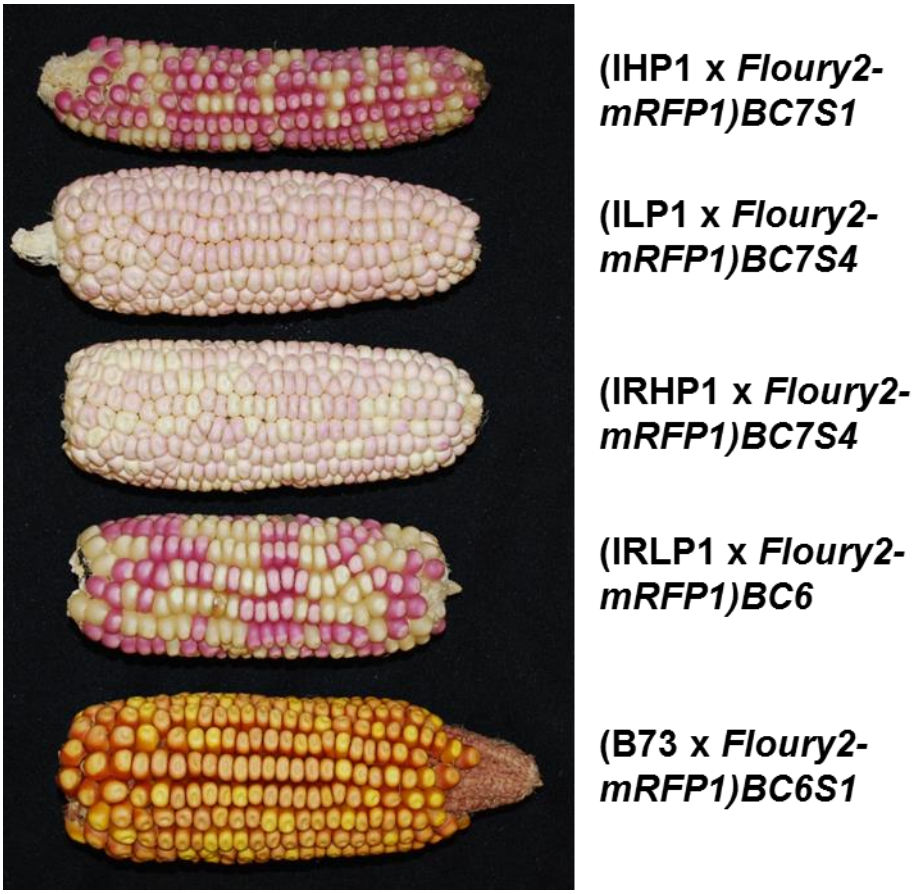


Figure 2.3. Ears produced by backcrossing *Floury2*-mRFP1 to the inbred IPS and B73.

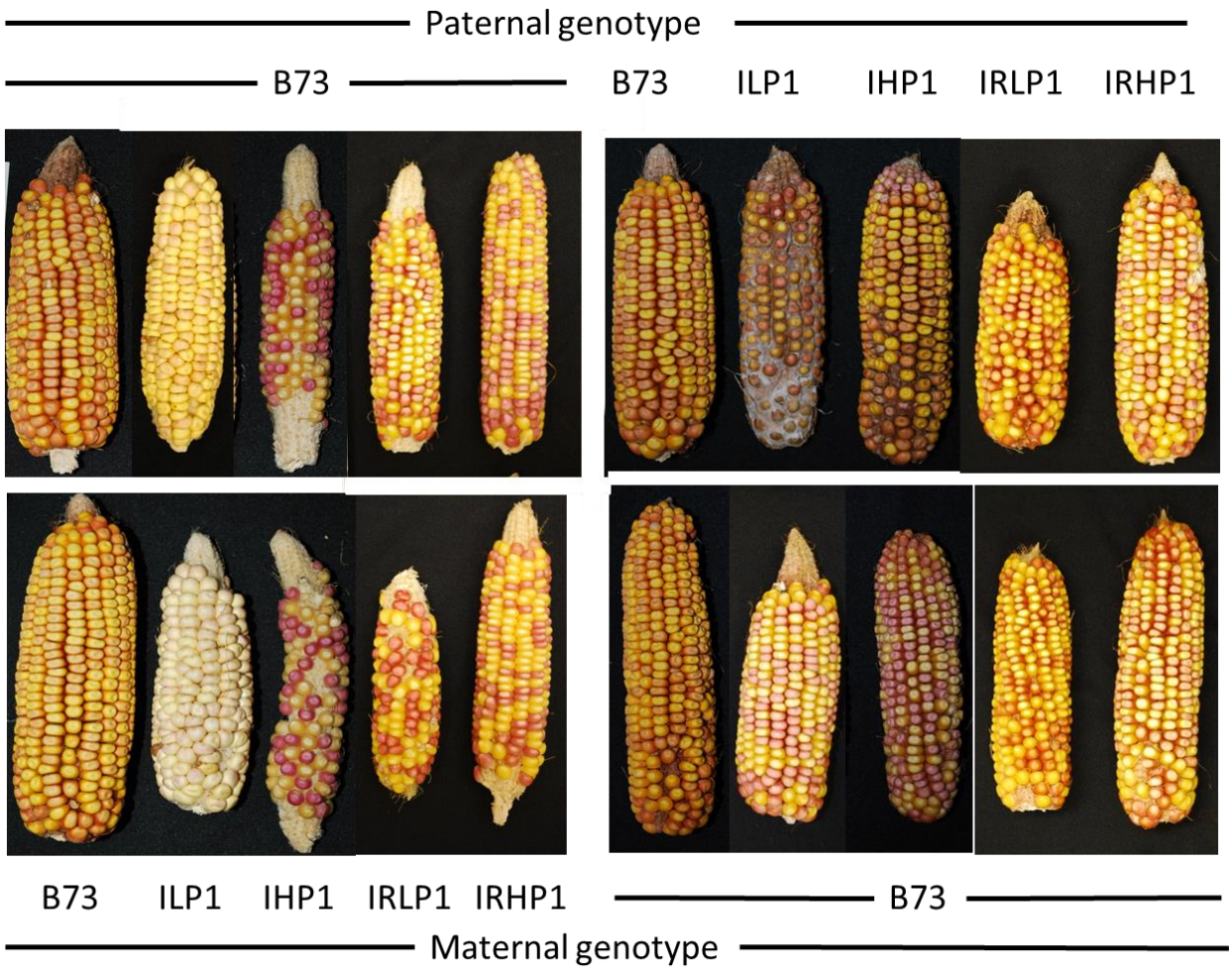


Figure 2.4. Ears produced from reciprocal crosses between the inbred IPS and inbred variety B73, where *Floury2*-mRFP1 is either transmitted through the female (top ears) or male (bottom ears). Maternal and paternal genotypes are indicated.

References

Alleman, Mary and Doctor, John. (2000) Genomic imprinting in plants: observations and evolutionary implications. *Plant Molecular Biology.* 43: 147-161.

Balconi, C., E. Rizzi, M. Motto, F. Salamini & R. Thompson (1993) The Accumulation of Zein Polypeptides and Zein Messenger-Rna in Cultured Endosperms of Maize Is Modulated by Nitrogen Supply. *Plant Journal,* 3, 325-334.

Bedell, J.A., M.A. Budiman, A. Nunberg, R.W. Citek, D. Robbins, J. Jones, E. Flick, T. Rohlfing, J. Fries, K. Bradford, J. McMenamy, M. Smith, H. Holeman, B.A. Roe, G. Wiley, I.F. Korf, P. D. Rabinowicz, N. Leakey, W.R. McCombie, J.A. Jeddeloh and R.A. Martienssen (2005) Sorghum genome sequencing by methylation filtration. *PLOS Biology*. 3(1):103-115.

Bhattramakki, D., M. M. Sachs & A. L. Kriz (1996) Expression of genes encoding globulin and prolamin storage proteins in kernels of Illinois long term chemical selection strains. *Crop Science,* 36**,** 1029-1036.

Bird, Adrian and Wolffe, Alan. (1999) Methylation-induced repression- Minireview Belts, Braces, and Chromatin. *Cell.* 99: 451-454.

Campbell, R., Tour, O., Palmer, A., Steinbach, P., Baird, G., Zacharias, D., and Tsien, R. (2002) A monomeric red fluorescent protein. *PNAS.* 99 (12): 7877-7882.

Church, J.B. (2008) Functional genomics of nitrogen use in maize. Ph.D. dissertation, University of Illinois.

Dembinski, E., Rafalski, A., Wisniewska, I. (1991) Effect of long-term selection for high and low protein content on the metabolism of amino acids and carbohydrates in maize kernel. *Plant Physiology and Biochemistry.* 29 (6): 549-557.

Gehring, M., Choi, Y., and Fischer, R. (2004) Imprinting and seed development. *The Plant Cell.* 16: S203-S213.

Hoener and DeTurk (1938) The absorption and utilization of nitrate nitrogen during vegetative growth by IHP and ILP corn. *J. American Society of Agronomy.* 30(3).

Lauria, M., Rupe, M., Kranz, E., Pirona, Raul, Viotti, A., and Lund, G. (2004) Extensive maternal DNA hypomethylation in the endosperm of *Zea mays. The Plant Cell.* 16: 510-522.

Lorenzoni C, Alberi, P., Viotti, A., Soave, C., Di Fonzo, N., Gentinetta, E., Maggiore, T and Salamini, F. (1978) Biochemical chracterization of high and low protein strains of maize. *In* Miflin and Zoschke ,eds, Carbohydrate and protein synthesis. Commission of the European Communities, Brussels, pp 173-197.

Lucas, C.J., H. Zhao, M. Schneerman S.P. Moose (2013) Changes in response to 110 cycles of selection for seed protein and oil concentration in maize. *In* Philip W. Becraft, ed, *Seed Genomics*. Wiley-Blackwell.

Lund, G., Ciceri, P. and Viotti, A. (1995) Maternal-specific demethylation and expression of specific alleles of zein genes in the endosperm of *Zea mays*. *Plant J*. 8: 571-581.

Mohanty, A., Luo, A., DeBlasio, S., Ling, X., Yang, Y. Tuthill, D.E., Williams, K.E., Hill, D., Zadrozny, T., Chan, A., Sylvester, A.W., and Jackson, D. (2009) Advancing cell biology and functional genomics in maize using fluorescent protein-tagged lines. *Plant Physiology*. 149: 601-605.

Moose S.P., Dudley J.W., Rocheford, T.R. (2004) Maize selection passes the century mark: a unique resource for 21st century genomics. Trends in Plant Science 9: 358-364.

Reggiani, R., and Soave, C. (1985) Factors affecting starch and protein content in developing endosperms of high and low protein strains of maize. *Genetica Agrarica.* 39: 221-232.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *PNAS* 81:8014-8018.

Salas, A. (2008) Regulation of zein transgene expression in response to long term selection for grain protein concentration in maize. M.S. thesis. University of Illinois at Urbana-Champaign.

Seebauer, J.R., S.P. Moose, B.J. Fabbri, L.D. Crossland and F.E. Below (2004) Amino acid metabolism in maize earshoots, implications for assimilate preconditioning and nitrogen signaling. *Plant Physiology,* 136: 4326-4334.

Singletary, G.W., D.C. Doehlert, C.M.Wilson, M.J. Muhitch, and F.E. Below (1990) Response of enzymes and storage proteins of maize endosperm to nitrogen supply. *Plant Physiology,* 94(3): 858-864.

Tsai, C.L., Dweikat, I. and Tsai, C.Y. (1990) Effects of source supply and sink demand on the carbon and nitrogen ratio in maize kernels. *Maydica* 35: 391-397.

Uribelarrea, M., Below, F.E. and Moose, S.P. (2004). Grain composition and productivity of maize hybrids derived from the Illinois Protein Strains in response to variable nitrogen supply. *Crop Science* 44: 1593-1600.

Uribelarrea, M., Moose, S.P., and Below, F.E. (2007) Divergent selection for grain protein affects nitrogen use efficiency in maize hybrids. *Field Crops Res*. 100: 82-90.

Wenck, A., C. Pugieux, M. Turner, M. Dunn, C. Stacy, A. Tiozzo, E. Dunder, E. van Grinsven, R. Khan, M. Sigareva, W. C. Wang, J. Reed, P. Drayton, D. Oliver, H. Trafford, G. Legris, H. Rushton, S. Tayab, K. Launis, Y. F. Chang, D. F. Chen & L. Melchers (2003) Reef-coral proteins as visual, non-destructive reporters for plant transformation. *Plant Cell Reports,* 22, 244-251.

Wrage, E. (2005) Characterization of the alpha-zein storage proteins of the Illinois maize long-term selection strains. M.S. thesis. University of Illinois at Urbana-Champaign.

CHAPTER 3. ASN-CYCLING AND ZEIN-SYNTHESIS CANDIDATE GENE STUDIES AND DISCOVERY OF NOVEL CANDIDATES FOR KERNEL COMPOSITION TRAITS

Introduction

Within ten cycles of selection of the Illinois experiment it was observed that, in addition to altered kernel composition traits, a number of other traits became altered (Hopkins, 1903; Smith, 1908). For example, all Illinois Protein Strains (IPS) demonstrated an inverse relationship between grain protein concentration and starch concentration. Due to the strong positive correlation between starch and yield, grain protein and yield are also inversely related. In this way, IHP contains the highest grain protein concentration, but the lowest starch concentration and yield. In contrast, ILP contains the lowest grain protein concentration, but the highest starch concentration and yield. On average, ILP contains only half of the protein as IHP, but two-fold more grain yield than IHP (Below et al., 2004). Grain protein and kernel size are also inversely related. Therefore, although IHP kernels contain twice as much protein as ILP kernels, because their kernels are much smaller the protein concentration is much higher than a two-fold increase over ILP (Below et al., 2004). Selection for high protein has also resulted in greater lodging and shorter plant height, and increases in successful germination and tillering (Woodworth et al., 1952). These traits are oppositely affected by selection for low protein, where the poor germination of ILP was one of the reasons it was discontinued, presumably due to a lack of nitrogen for the developing seeds.

Whole-plant nitrogen and carbon metabolism have also been altered in response to selection for grain protein concentration. Physiological changes affecting nitrogen metabolism were initially noted by Hoener and DeTurk (1938), who documented an

accumulation of more whole plant N in IHP compared to ILP and more carbohydrates in ILP. Several other studies attributed these observations to elevated N uptake, N assimilation and N remobilization from leaves to grain of IHP compared to ILP, and elevated C assimilation and remobilization of ILP (Lorenzoni et al., 1978; Reggiani and Soave, 1984; Tsai et al., 1990; Dembinski et al., 1991). Similar results were reported in hybrids between inbred IPS and a common elite tester (Uribellarrea et al. 2004 and 2007). Compared to ILP plants, IHP plants exhibit up-regulation of enzymes involved in ammonia assimilation, including higher levels of nitrate reductase in both leaves and roots, as well as downstream products (Dembinski et al., 1991; Lohaus et al., 1998; reviewed in Below et al., 2004). On the contrary, ILP plants exhibit higher levels of enzymes regulating starch biosynthesis, including ADP-glucose pyrophosphorylase, which is consistent with its increased starch production (Reggiani et al., 1985; Below et al., 2004). ILP also channels more sucrose to the grain, while IHP simply accumulates it in the stalk.

One of the most significant observations of prior studies is an elevated ratio of total amino-N contained in amino acids to sucrose in IHP tissues, including leaf, phloem sap and grain (Lohaus et al., 1998). These differences largely result from hyperaccumulation of Asn in IHP, which were 13-fold higher in leaves, 4-fold higher in seeds, and 3-fold higher in phloem, compared to the same tissues in ILP. These observations establish a strong correlation between Asn levels and seed protein concentration in the IPS, which has also been reported in other species, including Arabidopsis (Lam et al., 2003), soybean (Hernandez-Sebastiam et al., 2005), wheat (Barniex, 2007) and rye (Dembinski and Bany, 1991). Preferential accumulation of Asn

in such plants likely results from the molecule's high N:C ratio and neutral charge (2N:4C for asparagine compared to 2N:5C for glutamine or 1N:5C for glutamate), which makes it a stable molecule for N transport through the xylem and phloem and eventual storage within the endosperm. Increases in Asn content have been shown to be countered by simultaneous decreases in Gln, and it has been hypothesized that it is actually the ratio of Gln: Asn entering the cob that functions as a signal of vegetative N status for grainfill (Seebauer et al., 2004).

The observation of increased Asn in IHP plants indicates the priority to store N, which may be achieved through increased production or decreased breakdown of Asn, or both. The primary route for Asn synthesis in plants is through transfer of an amino acid group from Gln to aspartate (Asp) by the enzyme asparagine synthetase. The reverse process occurs through the release of stored ammonia from Asn by the enzyme L-asparaginase. The relative activities of the enzymes involved in the Asn-cycling pathway essentially modulate the C:N balance in the whole plant by serving as a switch that either directs N for storage in the form of Asn, or promotes growth and biomass accumulation by releasing the C and N tied up in Asn. Therefore, *asparagine synthetase* and *asparaginase* are strong candidates for upstream regulation of α-zein synthesis.

The *asparagine synthetase* (*AsnS)* gene family in maize is encoded by a family of four genes, but the *AsnS3* copy is the only copy expressed globally within the plant (Todd et al., 2008). The ortholog of the maize *AsnS3* gene in *Arabidopsis thaliana* (*ANS1*) has been shown to be down-regulated during the day by a bZIP transcription factor (*bZIP1*), whose expression is blocked by master clock control genes *CCA1* and

GLK1 (Gutierrez et al., 2008). Up-regulation of *AsnS3* expression during the night was also reported (Todd et al., 2008), and it is hypothesized that a putative maize ortholog of *bZIP1* may serve to regulate diurnal control. First, a highly homologous DNA binding sequence has been identified in the promoter of maize *AsnS3* (data unpublished). Secondly, mutant versions of this sequence in IHP but not other maize genotypes (ILP or B73) alter *AsnS3* expression in such a way that it is de-coupled from normal down-regulation by light, possibly by altering binding of the putative light regulated *bZIP1* ortholog. As a result, unlike other maize genotypes, *AsnS3* is expressed at high levels in IHP during the day (Church, 2008 and Lohaus et al., 1998). Combined with elevated expression at night, sum *AsnS3* expression is much higher in IHP compared to ILP. Global gene expression profiling studies in the Moose lab have identified a candidate gene annotated as containing a bZIP domain (GRMZM2G024851) that exhibits up-regulation in IHP versus ILP (data unpublished). This gene represents an additional candidate gene worth investigating here, and a molecular marker for this gene has also been developed.

The strong correlation between Asn and seed protein and the apparent decoupling of *AsnS3* from light regulation in IHP strongly suggests the importance of the Asn-cycling pathway in regulation of grain protein concentration. It is also known that OPAQUE2 (O2) (Schmidt et al. 1992) and PROLAMIN-BOX FACTOR1 (PBF) (Vicente-Carbajosa et al. 1997; Marzabal et al. 2008) transcription factors regulate α-zein mRNA expression, as described in detail in chapter one. Therefore, genes in both the Asn-cycling and zein synthesis pathways represent strong candidates. Three main experiments are employed here to test their biological roles (**objective 2**), and these

studies make use of the inbred Illinois Protein Strains (IPS), the populations at cycles 65 and 100, and the Illinois Protein Strain Recombinant Inbreds (IPSRIs).

First, in response to selection, the IPS populations are predicted to exhibit preferential accumulation of favorable alleles that are inconsistent with Hardy Weinberg Equilibrium (HWE). It is possible to monitor changes in allele frequency of candidate genes between cycles 65 and 105 of the IPS. Fixation of allele frequency at cycle 65 or 105 or dramatic shifts between the interval may signify both if and when these loci were targeted by selection. This type of analysis has already been documented for the zein loci, as determined by RFLP analysis by Wrage (2005). However, due to the large number of zein loci, the results are difficult to interpret. In order to simplify the interpretation of these data, it is reanalyzed here by defining six major zein loci haplotypes in the IPS. Similar analyses were conducted for candidate genes in the Asn-cycling and zein synthesis pathways.

Secondly, because long-term selection has specifically altered α-zein protein accumulation in the IPS (Bhattramakki et al., 1996), large differences in mRNA expression of α-zein genes and any candidate gene thought to regulate zein expression are predicted. The collective results of two studies have already confirmed up-regulation of *AsnS3* expression and protein abundance in IHP in both leaves and roots (Lohaus et al., 1998 and Church, 2008), which is consistent with its elevated Asn levels. However, a more thorough analysis is needed to fully understand the roles of additional candidates. To document mRNA expression in the IPS, qRT-PCR was conducted on the inbreds and cycles (65 & 105) for zein genes and putative regulators.

Third, the genetic effects of candidate genes can be empirically tested when associated with kernel composition phenotypic data collected on the IPSRI mapping population. This type of candidate gene association analysis has been conducted for candidate genes involved in starch biosynthesis, where positive associations were detected for starch composition (*brittle endosperm2, shrunken1 and shrunken2*), and starch pasting properties and amylose levels (*amylose extender1* and *shrunken2)* (Wilson et al. 2004). Molecular markers for *AsnS3, ASNase,* and the putative ortholog of *At. bZIP1* for the IPS have already been developed by members of the Moose lab and were available for use here. SSR molecular markers for *O2* (Hartings et al., 1995) and *PBF* (Vicente-Carbajosa et al., 1997) have also been developed. Due to the small size of the *O2* polymorphism, it was necessary to first clone and sequence *O2* in IHP1 and ILP1 genotypes in order to accurately characterize alleles, and these results are presented first.

The significance level of candidate genes can be determined by single marker analysis. However, this type of analysis lacks empirical generation of a background significance threshold, making it difficult to distinguish true from false positives. Wilson et al. (2004) empirically tested for false positives by associating ten genes in unrelated pathways with starch composition traits. To provide negative control molecular markers, high-throughput genotype-by-sequencing data was generated on the IPSRIs. This allowed for generation of genome-wide SNP markers (described below), which should determine an appropriate background significance level and reduce false positive results. In addition, genome-wide SNP markers will simultaneously allow for genome-wide association studies for novel candidate gene discovery (**objective 4**). A

positive control gene was also desired for the genome-wide association analyses. *Glossy15* was chosen for this purpose because a phenotype similar to that observed in *glossy15* mutations (adult instead of juvenile leaf waxes beginning with leaf 3, earlier onset of leaf macrohairs) was observed to segregate among the IPSRIs throughout their inbreeding, and is also present in individuals from cycle 70 of Illinois Low Protein. Furthermore, testcrosses of some of the IPSRIs with the glossy leaf phenotype produced glossy15 mutant progeny when crossed to a known glossy15 mutant line.. Mapped to chromosome 9, *Glossy15* controls the juvenile-to-adult phase transition in maize, which is manifested by the abbreviated expression of a series of juvenile leaf traits, including the presence of epicuticular wax (Moose and Sisco, 1994). Several molecular markers have been developed for *Glossy15*, including an SSR marker within the promoter, *umc1688*, and an indel in the 3' UTR, *umc1691*. Another recent study of a large and diverse population of maize inbreds also identified *Glossy15* as a major QTL controlling the number of leaves producing juvenile leaf wax (Hirsch et al., 2014). Significant associations between markers in the *Glossy15* locus on chromosome 9 and the *glossy15* mutant phenotype are thus expected.

The physiological extent to which divergent selection has altered whole plant N and C metabolism in the IPS has led to the hypothesis that many genes have also been affected (Moose et al., 2004). This is confirmed by the results of prior genetic mapping studies utilizing the advanced random-mated IHP x ILP population (cycle 70) (Dudley et al., 1977), which identified many QTLs having small phenotypic effects (Goldman et al., 1993; Dijkhuizen et al., 1998; Dudley et al., 2004; Clark et al., 2006; Dudley et al., 2007). However, these studies were complicated by population structure arising from

the use of multiple parents in the initial cross (5-7 IHP crossed to 5-7 ILP individuals) and the phenotyping of 200 segregating RM7S1 families, rather than inbred lines. The Moose laboratory has since generated inbred lines by selfing the 500 RM7S1 six generations to create an RM7S7 population, the Illinois Protein Strain Recombinant Inbreds (IPSRIs). The creation of inbred lines eliminates the complexity associated with segregating families, while still taking advantage of reduced LD from advanced inter-mating (Lee et al., 2002; Clark et lal., 2006; Dudley, 1994; Dudley et al., 2004).

However, due to the high frequency of recombination events and reduced LD in advanced intermated populations, a large number of markers is required for QTL mapping. The maximum number of markers used by previous QTL mapping studies with the advanced random-mated IHP x ILP population was 500 SNP markers (Dudley et al., 2007), but this number is likely insufficient to detect with high probability the majority of marker-trait associations. Therefore, if coupled with high-throughput genome-wide molecular markers, the IPSRIs should permit precision QTL mapping and genome-wide SNP-trait associations for discovery of novel candidates.

The recent development of low-cost high-throughput sequencing technologies provides a number of options for genome-wide marker development on the IPSRIs. Genotyping by Sequencing (GBS) involves genome complexity reduction using restriction enzymes and offers the ability to obtain a large number of SNP markers for approximately $30 per sample (Elshire et al., 2011). Unlike array based genotyping methods, which are subject to ascertainment bias, GBS has recently been applied to diverse maize populations. Using the genotyping-by-sequencing method of Elshire et al. (2011) and SNP-calling method of Glaubitz et al. (2014), SNP markers were

developed for the IPSRIs. Inbreds IHP1, ILP1, IRHP1, IRLP1 and B73 were also included.

The IPSRI mapping population was phenotyped for three main traits. These included total grain N concentration by combustion analysis from plants grown in summers of 2008 and 2009 and near infrared reflectance (NIR) of grain produced in 2011 and 2012. Additionally, a novel phenotype was developed that tracks expression of a single 22-kD α-zein gene, *Floury2,* through the use of a red fluorescent protein (mRFP1) promoter reporter (Mohanty et al. 2009). Transgene construction and phenotype characterization are described in detail in chapter two. A method for precise quantification of *Floury2*-mRFP1 expression that uses direct and non-destructive imaging of transgenic ears was developed, and the phenotyping platform is described here (**objective 3**). The combination of genotypic and phenotypic data collected here will serve three purposes: to provide negative control markers for candidate gene-phenotype associations, to conduct genome-wide SNP-trait association studies to discover novel candidates, and to build a linkage map for purposes of QTL linkage mapping.

Materials and Methods

Plant Material

Fifteen Illinois Protein Strain Recombinant inbred (IPSRI) seeds were planted in 12 foot plots in 30 inch rows at the Department of Crop Sciences research and education center in Champaign, Illinois during the 2008, 2009, 2011 and 2012 growing seasons. In 2008 and 2009, approximately five IPSRI plants were self-pollinated per genotype,

the ears were hand shelled, and bulk phenotypic measurements were collected. In 2011 and 2012, two rows of B73:*Floury2*-mRFP1 seeds were planted as pollen donors for every ten rows of IPSRIs planted. These crosses were necessary for expressing the transgene in the IPSRIs. One of the two B73:*Floury2*-mRFP1 rows was delayed ten days due to observed differences in flowering time between the IPSRIs and B73:*Floury2*-mRFP1 plants. Up to five IPSRI plants were cross-pollinated with B73:*Floury2*-mRFP1. Ears were hand shelled individually, and phenotypic measurements were collected on an individual ear basis, as described in the phenotypic trait measurement section. DNA was already available for individuals from the IPS, cycles 65 and 100 (Wrage, 2005); therefore, these populations did not have to be re-planted for analyses conducted here.

Extraction of Zeins and SDS-Polyacrylamide Gel Electrophoresis (PAGE)

More than 50 kernels from 24 day after pollination (DAP) ears were sampled and pulverized to a fine meal. Complete solubilization of proteins were isolated from 100 mg of meal in 1.0 mL of 0.0125 M sodium borate (pH 10.0), 1% SDS, and 2% 2-mercaptoethanol. Zeins were extracted overnight in 70% (v/v) ethanol with constant shaking at 37°C. After centrifugation for 15 min at 12,000 rpm, the supernatant was collected, vacuum dried, and stored at 4°C until use (Wallace et al. 1990). SDS-polyacrylamide gradient gels (7.5-18%, w/v) were prepared. Protein samples were diluted in Laemmli sample buffer and boiled for 3 min to denature before loading. Gels were run at room temperature at a constant current of 15 mA until the dye front migrated through the stacking gel, and then at 25mA through the resolving gel. Gels

were stained with Coomassie Brilliant Blue R250 overnight, and de-stained in 40% (v/v) methanol and 10% (v/v) acetic acid for at least 8 hours.

Genetic Markers

Optimal primers which specifically amplified the targeted DNA sequence of the gene of interest were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi). BLAST searches confirmed the total gene specificity of the primer sequences and BLASTN searches showed the absence of polymorphisms at the primer site. The sequences below are oriented in the 5' to 3' direction.

The *AsnS3* (GRMZM2G053669: *asparagine Synthetase3)* indel and *asparaginase* (CAPS and indel) markers were developed by Farag Ibraheem and Han Zhao, respectively, to identify and amplify polymorphisms between IHP1 and ILP1 by PCR**.** The *AsnS3* primers anneal within the promoter region of *AsnS3* on chromosome 1S and result in 2 bands for IHP1, 498 and 778 bp and one band for ILP1, 557 bp. *AsnS3* primer sequences are as follows: Forward: CTCAACTCATCGGCACAGACTTGCATC ; Reverse: TCGAATTTATCCTTTCTACAACCCCAATC.

The ASNase (GRMZM2G082032: *L-asparaginase)* CAPS marker primers anneal the third exon of *L-asparaginase* on chromosome 2, and the sequences are as follows: Forward: CCGTCATGGAGTACAAGGGCCT; Reverse2: CTTATCCTCCAGTTTATTG OR R1 GATGAGATGATGAACAAC. The marker requires cleavage by the Hpy188I

restriction enzyme (NE Biolabs), which results in 2 bands of 73 bp and 241 bp for IHP1 and one band of 314 bp for ILP1.

The ASNase3UTR (GRMZM2G082032: *L-asparaginase)* indel marker was developed by Yuhe Liu to amplify a 33-bp deletion in the 3'UTR region of the *Asparaginase* gene on chromosome 2. Primer sequences are as follows: Forward: GGAGGTCGGCATCTGGAGTGA; Reverse: AAACACATGGCAATCGCAGGATGG.

The *umc1066* (GRMZM2G015534: *Opaque2*) SSR marker is described by Hartings et al. (1995). *O2* primers amplify a hypervariable region in the N-terminal part of *O2* on chromosome 7S that varies in the number of Pro-Gln repeats.
Primer sequences are as follows: Forward: ATGGAGCACGTCATCTCAATGG; Reverse: ATGGAGCACGTCATCTCAATGG.

A second *Opaque2* marker used for cloning is described by Henry et al. (1997). The O2-711 SSR marker contains the hypervariable region described above. O2-711 primer sequences are as follows: Forward (U): GCAGGGAGGCACAGCAAG; Reverse (L): AGGGAAGAAGAGCGAGCAGT.

The *umc1065* (GRMZM2G146283: *Prolamin-box factor1*) primers amplify an SSR in the 3' end of the *prolamin-box factor1* gene on chromosome 2L that varies in number of Asn repeats (Vicente-Carbajosa et al., 1997). *Umc1065* primer sequences are as follows:

Forward: ACAAGGCCATCATGAAGAGCAGTA; Reverse: CACGGTCTGGCACACTAACCTTAT.

The bZIP primers amplify three sequences with high homology that are believed to be paralagous gene copies. Two copies reside on chromosomes 3 (GRMZM2G024851 and GRMZM2G143290) and the other on chromosome 6 (GRMZM2G116494). Due to high sequence homology, attempts to develop primers that specifically amplify a single copy were not succesful. bZIP primer sequences are as follows: Forward: TTGGGAATGGCCTGATACTC; Reverse: CCATGGTGAATAAAGCTGGAA.

The *umc1943* SSR marker is not associated with any gene model, but is between position 5,493,381 and position 5,492,675 (B73ref v2) and lies within the 19-kD α-zein (z1A) cluster on chromosome 4S (MaizeGDB). Primer sequences are as follows: Forward: GTGCTGCAGAATTCAACTCCTTC; Reverse: ACCATTTCTGCGTTTCCACAGT.

The *umc2150* SSR marker is not associated with any gene model, but is between 5,375,300 and position 5,380,200 (B73ref v2) and lies within the 19-kD α-zein (z1A) cluster on chromosome 4S (MaizeGDB). Primer sequences are as follows: Forward: GTTGTTCACTTTCCAAAACCCTTG; Reverse: GCCTTGTGCTTCTTGGAGTGTT.

The *p-bnlg421* SSR marker is not associated with any gene model, but is between 20,464,471 and position 20,639,417 (B73ref v2) and lies within the 22-kD α-zein (z1C) cluster on chromosome 4 (MaizeGDB). Primer sequences are as follows: Forward: AGCCAGTTGCCCAGCATCT; Reverse: GGGGCAAGGACTTGTCGGT.

The *umc1688* (*Glossy15*) SSR marker is found within the promoter of *Glossy15.* Primer sequences are as follows: Forward: TCGTCTAAACTGCATAAAAGGGGA; Reverse: ACGGAGATAGATGCACACAAACAC.

The *umc1691* (*Glossy15*) indel marker is found in the 3' UTR of *Glossy15.* Primer sequences are as follows: Forward: AGCAGTAGCCGCAAGCAGAG; Reverse: ATCTGGAGCTGCGTGCTGTC.

PCR Protocol

2.5 μL 10x Standard Taq Reaction Buffer (NE Biolabs), 2μL 10mM dNTPs (Biorad), 0.2μL homemade taq polymerase, 1μL Forward & 1μL Reverse 10nM primers, 100ng DNA, and 16.8μL dH20, for a total volume of 25μL, were used for PCR. The PCR amplification profile used an initial denaturation step of 95°C for 2 mins, followed by 40 cycles of 95°C for 30 secs annealing at 58-62°C, depending on primer melting temperatures, for 1 min, and extension at 72°C for 1 min.

Opaque2 Cloning and Sequencing

DNA was extracted from tissue samples following the method described in the DNA extraction section. Then DNA samples were purified using the QIAquick PCR Purification Kit following the manufacturer protocol. Cloning was conducted using the pGEM-T and pGEM-T Easy Vector Systems by Promega. Manufacturer protocol was followed with a few minor modifications. First, the ligation protocol was modified by decreasing reagent volumes by 50%. Secondly, the transformation protocol was modified by the addition of a second plating volume, 400μL in addition to the suggested 100μL volume. Sequencing was performed by the Keck Center at University of Illinois. Alignment was conducted using BioEdit and Vector NTI.

RNA Isolation

Total RNA from 16 DAP developing seeds of each genotype was isolated using Trizol LS Reagent (Invitrogen, Carlsbad, CA) due to the high starch concentrations in the seeds, then was treated with DNase I and purified using a RNeasy mini kit (Qiagen, Valencia, CA) as described in the manufacturers' protocols. The RNA concentration, quality and integrity were assessed with the ND-1000 spectrophotometer (Nanodrop, Wilmington, DE) and agarose gel electrophoresis analyses.

Quantitative Real Time PCR

Quantitative real time PCR on inbred IPS was performed with 2 biological replications. First-strand cDNA was generated from 5 μg of total DNase-treated RNA using SuperScript™ III Reverse Transcriptase (Invitrogen) and oligo(dT) primer according to

manufacturer's instructions. SYBR Green PCR Master Mix (Applied Biosystems Inc., Foster City, CA) was used in the two step of the RT-PCR for relative quantitation of the cDNA following the manufacturer's protocol. Amplification and detection were performed on a DNA Engine Opticon 2 (Bio-Rad Laboratories, Hercules, CA). Primer sequences are listed in the Genetic Markers section. BLAST searches confirmed the total gene specificity of the primer sequences and BLASTN searches showed the absence of polymorphisms at the primer site. The efficiency of amplification (E) was determined according to the following equation: E = 10(–1/slope), using the slope of the regression curve obtained by plotting C_T values. Only E above 90% was used for further data analysis. The validation experiments were carried out in triplicate. Melt curve analysis was conducted following each reaction to confirm the presence of only a single product of the reaction. Negative control reactions were performed for a selection of samples using RNA that had not been reverse transcribed to control for the possible presence of genomic contamination. Normalization of the Ct values was carried out by subtracting the C_T values of the control gene, *GAPDH*, yielding ΔC_T values for each gene. Values for $\Delta\Delta C_T$ were then derived by subtracting each ΔC_T by the ΔC_T value obtained from the same gene in the calibrator sample, B73. Fold changes of inbred IPS gene transcripts were then calculated relative to the corresponding transcript from B73. The same protocol was used for cycle 65 and 105 individuals with the exception that these represented pools of DNA from approximately 24 individuals for each cycle-strain combination.

Phenotypic Trait Measurements

For NIR phenotypes, approximately 125 kernels were ground to a fine flour using a grinding mill. Kernel composition traits, including the percentage of protein (N), starch, and oil, were measured using the DICKEY-john Instalab 600 near-infrared reflectance (NIR) analyzer. This method is discussed in detail in chapter one. For the days to flower phenotype, the number of days it took for approximately half of the plants within a row to reach anthesis was recorded for the IPSRIs in 2012, and is referred to as the 'days to flower' phenotype. For the *glossy15* phenotype, juvenile leaves were scored in 2009 for the last leaf expressing wax. Plants lacking wax on leaf 4 or 5 were scored as having the characteristic glossy leaf mutant phenotype and were scored as *glossy15* mutants. Among the 138 IPSRIs, seven were fixed for the *glossy15* mutation.

Development of the Floury2-mRFP1 Precision Phenotyping Method and Phenotypic Trait Measurements

A method for quantification of *Floury2*-mRFP1 expression was developed that uses direct and non-destructive imaging of transgenic ears. Here, we introduce the phenotyping platform as it is applied to SNP-trait associations using the segregating IPSRI population. IPSRI x B73:*Floury2*-mRFP1 ears were harvested and wrapped tightly in pollination bags, and these were stored in black plastic garbage bags to prevent photo-bleaching of the mRFP1 protein by sunlight. The ears were then numbered and the whole ears photographed using a high quality 48-bit Nikon DX camera under standard lighting conditions. 48-bit images were saved as tifs.

The digital image processing software program Axiovision (version rel 4.8) was used to select areas of the kernels to be measured. Areas were chosen based on several criteria. First, they were to be free of glare due to the camera flash and free of shadows from other kernels. Secondly, only disease-free kernels were chosen for measurement. Thirdly, the dent region of the kernel was avoided due to the observable difference in color, likely due to the fact that cells in this area had died. Lastly, approximately equal numbers of kernels were measured from the base, middle and tip of the ears. The color data generated from Axiovision is split into red, green and blue channels, and all three are collected automatically. However, a ratio of red:green was used as the final phenotypic measurement because it exhibited the strongest correlation with protein concentration. 36 measurements (3 per kernel x 12 kernels) were collected on pink kernels for each ear for 2011. A bootstrapping procedure (sample replicate number = 1,000, with replacement) was performed in SAS v.9.3 to determine if fewer measurements would generate similar results when an analysis of variance (ANOVA) was performed. It was determined that 12 measurements (1 per kernel x 12 kernels) did not significantly alter sums of squares or R^2 values. Therefore, only 12 measurements were collected for ears generated in the 2012 field season following the same criteria as listed above. Two ears were measured per genotype per year for 2011 and 2012. After images were taken, approximately 125 whole kernels were ground to a fine flour, and NIR was performed on the same ears for which *Floury2*-mRFP1 data was first collected.

Variance Component and Correlation Analysis

Analysis of variance (ANOVA) was conducted in SAS (Version 9.3) using the procedure PROC GLM. Year, genotype and earrep (ear replication) were entered into the model as classification terms. The relative contribution of various components, including genotype, year, ear replication (earrep), and the genotype by year interaction, were determined relative to the total variance of each trait studied using the procedure PROC VARCOMP. The ANOVA data was also used to calculate broad sense heritabilities for each trait. Pearson correlation analysis was conducted in JMP Genomics (version 7.0) using the procedure Multivariate.

DNA Extraction

The DNA extraction method is an adaptation of the method of Saghai-Maroof et al. (1984). Fresh leaf tissue was collected from V8 plants in 15mL round bottom tubes, lyophilized, and ground to a fine powder using steel beads and a geno grinder at 500 RPM for 1 min. 300mg of lyophilized ground tissue was weighed and transferred to new canonical bottom tubes. 9mL of fresh /CTAB extraction buffer was added, mixed by inverting, and incubated in a hot water bath at 65 degrees C for 60 min with gentle inverting on 10 min intervals. After incubation, samples were allowed to cool for 10 min. 4.5mL of chloroform:isoamyl alcohol (24:1) was added, and the samples were rocked gently for 5 min. Then samples were centrifuged for 10 min at 2800 rpm at room temperature. The top aqueous layer was removed into new 15mL polypropylene tubes containing 50uL of 10mg/mL chloroform/isoamyl alcohol layer, mixed well by inverting tubes, and incubated for 30 min at room temperature. 4.5mL of chloroform:isoamyl

alcohol (24:1) was added, and the samples were rocked gently for 3 min. Then samples were centrifuged for 10 min at 2800 rpm at room temperature. The aqueous layer was carefully transferred to a tube containing 6mL of isopropanol, making sure not to remove the band in between the top CTAB layer and the chloroform/isoamyl alcohol layer, and gently inverted to mix. The precipitated DNA was gently wound around sterile glass rods and transferred to 5 mL tubes containing 2 mL of 80% EtoH, and left to wash for 20 min. The samples were then centrifuged for 10 min at 2800 rpm. Supernatant was poured off. 2 mL of 80% EtoH was added back to the tubes, which were incubated for 20 min at room temperature. The tubes were then vortexed gently to loosen the pellets. The DNA was transferred to microfuge tubes containing 100 uL 1X TE, and the samples were rocked gently overnight on a rotator to dissolve the DNA. The following day, samples were spun for 10 min at 10,000 rpm and the supernatant transferred to fresh microfuge tubes. Samples were nano-dropped using a spectrophotometer.

Marker-Trait Associations

GBS was conducted by the Institute for Genomic Diversity at Cornell University using the methods of Elshire et al. (2011). SNP calls were made by Ed Buckler's lab using the pipeline described in Glaubitz et al. (2014). Approximately 955K unimputed SNPs were identified in the July 2013 build, but only about 70K (69,515) contained 90% or less missing data in the IPSRIs, were bi-allelic, and scored in at least 100 of the 138 IPSRIs. Additional filtering procedures described in the QTL Linkage Mapping section were also applied to generate a high confidence marker data set. The SNP-trait

associations produced very similar results using either the high confidence or the 70K SNP data sets, so only results using the 70K data set are presented. Analyses were conducted in JMP Genomics v.7.0 using a regression testing for linear trend of marker alleles. The days to flower phenotype was entered as a fixed effect. Because prior analysis determined a lack of population structure (see chapter one), it was not necessary to include covariates, such as the K or Q matrix. SNP filtering was conducted using R statistical software and JMP Genomics v.7.0. The list of annotations presented here was built from the phytozome.org annotation file by Jennifer Arp. Informative maize annotations were maintained, but for GRMs with no available maize annotation, orthologous annotations were used for either *Oryza sativa* or *Arabidopsis thaliana.* Orthologous annotations are preceeded by "Os:" or "At:" to indicate that they are not the true maize annotation.

QTL Linkage Map Construction

The 70K SNP dataset described in the previous section was filtered to generate a high confidence marker data set for linkage map construction. Because the expected segregation ratio of markers in a RIL mapping population is 1:1, the expected minor allele frequency (MAF) is 0.5. According to a Chi-Square analysis (α=0.01), markers with an observed MAF of 0.37 or greater do not significantly deviate from the expected MAF of 0.5. However, overlap is expected with markers segregating in a1:2 ratio. According to a Chi-Square analysis (α=0.01), markers with an observed MAF of 0.43 or greater significantly deviate from the expected MAF of 0.33 for markers with a 1:2 segregation ratio. Thus, only markers with a MAF greater than or equal to 0.43 and less

than or equal to 0.5 were included, thereby eliminating overlap with markers segregating in a 1:2 ratio. The SNPs were also filtered for percentage of missing data, where markers with a proportion missing greater than 10% were discarded. Finally, the SNPs were also required to be fixed for different alleles between inbreds IHP1 and ILP1. Individual IPSRIs were filtered for percentage of missing data, where individuals genotyped for less than 50% of loci were discarded. Individuals with greater than expected heterozygosity were also discarded.

Marker grouping and linkage map construction were performed using the maximum likelihood method (independence LOD = 6.0) in Joinmap 4.1 software (J.W. Van Ooijen, 2006). Identical SNPs were removed in JoinMap 4.0 prior to linkage map construction. Candidate genes exhibiting segregation distortion were removed. Linkage groups with fewer than five SNP markers were also excluded. Sixty-seven groups contained greater than or equal to five SNPs, and these could be manually ordered based on chromosome and basepair positions of the SNPs within the linkage groups. Linkage groups were manually broken if they displayed locally large gaps in map distance that corresponded with non-sequential basepair SNP coordinates on either side of the gap. These groups were then re-ordered manually among other linkage groups according to SNP basepair positions. To stitch together the linkage groups within a chromosome, it was necessary to estimate the map distances across the gaps, which could be achieved by dividing the difference in basepairs of markers on either side of the gap by the average physical distance per map unit for that chromosome. The average physical distance per map unit was calculated by subtracting the basepair coordinates of the first marker from the basepair coordinates of

the last marker on the chromosome and then dividing by the total map units (sum of all linkage groups) of that chromosome.

Results

Cloning and Sequencing of *Opaque2* in IHP and ILP

The O2-711 F and R primers amplify a 711 bp fragment that contains the hypervariable region of *O2*, which varies in the number of Proline-Glutamine di-peptide repeats (Henry and Damerval, 1997). This 711-bp region was amplified and cloned into the pGEM-t Easy Vector system. Cloned DNA was then amplified using the *umc1066* primers described in Hartings et al. (1994), which amplify a smaller fragment of the 711 bp fragment, but still contain the hypervariable region. The PCR product was run on an Agarose gel to confirm the presence of the appropriate fragment. Clones of IHP1 and ILP1 were sequenced by the UIUC Keck Center. A sequence alignment was performed in BioEdit, and the number of di-peptide repeats was compared to the banding patterns. Three bands slightly different in size were observed of approximately 145 bp, which is the expected product size using the *umc1066* primers. Band sizes were compared to corresponding sequence data, and agreement in size and di-peptide repeat number was observed; the highest band corresponded to the greatest number of repeats (n=5 repeats), and the smallest band corresponded to the least number of repeats (n=3 repeats). The intermediate band had n=4 repeats. IHP samples had 3-rt allele, and ILP samples the 4-rt allele.

SDS-PAGE Analysis of the Inbred Illinois Protein Strains

To document zein protein accumulation in the IPS populations, zein was extracted and pooled from 24 individual plants from cycles 65 and 100 and separated by SDS-PAGE (Figure 3.1). Cycles 65 and 100 were analyzed to represent dynamic changes over a 35 year period, and Illinois High Oil (IHO) and Illinois Low Oil (ILO) were included as unselected controls. As expected, abundance of 19-kD and 22-kD α-zein protein increased in IHP and IRLP but decreased in ILP and IRHP during this period. A slight decrease in protein was observed in ILO, and a slight increase was observed in IHO, consistent with the slightly positive correlation between protein and oil concentrations (Figure 1.1). These results were consistent with zein abundance in the inbred IPS (data not shown). The accumulation of the other classes of zeins, including the 14- and 16-kD β-zeins, the 27-kD γ-zeins and the 10-kD δ-zeins did not appear to be altered dramatically, nor was the abundance of non-zein proteins (data not shown).

Expression Profiling of Candidate Genes in the Illinois Protein Strains

To investigate mRNA changes of candidate genes hypothesized to have been targeted by long-term selection, an initial transcript profiling of the inbred IPS was conducted. These included a combination of microarray and qRT-PCR approaches. For candidates in the zein pathway, expression was assayed in seeds harvested at 16 days after pollination (DAP), the peak developmental stage for zein gene expression. Due to their extremely high sequence homology, designing RNA expression assays specific to individual zein genes is problematic. However both microarray probes and qRT-PCR primers have been designed that specifically monitor the major zein

subfamilies. qRT-PCR results of the inbred IPS are plotted in Figure 3.2. IHP1 exhibited up-regulation α-zein subfamilies Z19α, Z22α, and Z50γ compared to the other genotypes, whereas ILP1 exhibited down-regulation, relative to the B73 inbred line (Figure 3.2A). IRLP1 and IRHP expression was intermediate, similar to B73, and consistent with the changes in zein accumulation following reverse selection. Prior microarray studies by the Moose lab indicated that other zein families, including the Z27γ, Z15β and Z10δ zeins, were expressed at similar levels in all genotypes (data unpublished). qRT-PCR of the Z15β zeins confirms this result (Figure 3.2A). Analysis of *O2,* a transcriptional activator of Z22α genes, indicated no differences in the five inbreds assayed, except for reduced expression in ILP1 (Figure 3.2B). However, *PBF* expression patterns followed those of the α-zeins with lowest expression in ILP1, highest expression in IHP1, and intermediate expression in B73, IRHP1 and IRLP1.

While analysis of the inbred IPS provided a snapshot of expression variation at cycle 90 of the selection experiment, the second analysis investigated the dynamic changes over time by assessing transcript abundance (qRT-PCR) in pooled samples of individuals from IHP and ILP populations at cycles 65 and 105. Illinois High Oil (IHO) served as a control in the subsequent studies. Like before, expression was assayed in 16 DAP seeds for zein pathway candidates. For Asn-cycling candidates, expression was assayed in leaves at the vegetative tasseling (VT) stage of development, directly before grainfill begins. qRT-PCR results of the IPS populations at cycles 65 and 105 are plotted in Figure 3.3. Again using primers specific to each subfamily, Z22α and Z19α expression patterns were similar to those observed for the inbred IPS and consistent with known changes in protein accumulation over this 40-year period. For

this reason, only Z22α-FL2 is shown here (Figure 3.3A). By cycle 65, Z22α-FL2 expression was significantly higher in IHP compared to the other protein selections, but these differences were magnified by cycle 105, indicating a strong correlation between zein expression and protein accumulation. Expression analysis of the *O2* and *PBF* regulatory factors (Figure 3.3 B and C) revealed little correlation with known levels of zein accumulation at cycle 65. In fact, *PBF* expression was twice as high in ILP compared to IHP. However, between cycles 65 and 105 *PBF* and *O2* expression increased in IHP and decreased in ILP. Thus, by cycle 105 their expression patterns mirrored those of Z19α and Z22α genes and corresponding protein accumulation in the strains. Significant differences in *AsnS3* and *ASNase* expression were observed between IHP and ILP by cycle 65, but in opposite directions. While IHP demonstrated up-regulation of *AsnS3* (Figure 3.3D), ILP demonstrated up-regulation of *ASNase* (Figure 3.3E). Interestingly, expression patterns of the putative *bZIP1* ortholog closely followed those of *AsnS3* (Figure3.3F).

Allele Frequency Variation of Candidate Genes in the Illinois Protein Strains

Selection for grain protein concentration in the Illinois selection experiment has specifically altered α-zein protein concentration and α-zein expression in the IPS. To test for changes in allele frequency that may underlie these phenotypic changes, it was possible to survey and analyze allele frequencies of the strains spanning a forty year period. Wrage analyzed approximately twelve 22-kD zein loci using an RFLP analysis (Wrage, 2005), but these data were reanalyzed here to identify major haplotypes.

Similar analyses were conducted for additional loci, including candidate genes in the Asn-cycling and zein synthesis pathways.

Molecular markers were available for *O2, PBF, AsnS3 and ASNase*. For the *O2* gene, a region hypervariable for the number of proline-glutamine dipeptide repeats has been described by Hartings et al. (1995). This region was PCR amplified, cloned, and sequenced in IHP and ILP individuals, which revealed the presence of three alleles having 3 to 5 proline-glutamine dipeptide repeats, as described earlier. For PBF, an SSR marker was developed that varies in the number of Asn repeats in the 3' coding sequence (Vicente-Carbajosa, 1997). Three alleles having 11, 16 or 17 repeats were identified in the Illinois Protein Strains. Molecular markers for *AsnS3* and *ASNase* were developed by Farag Ibraheem of the Moose lab and were available for use here. The *AsnS3* marker distinguishes a 260-bp insertion in the IHP1 promoter, and the *ASNase* CAPS marker amplifies a 314 bp fragment that when cleaved by Hpy188I produces 2 bands of 73 bp and 241 bp for IHP1 and one band of 314 bp for ILP1.

Zein haplotype and candidate gene allele frequencies were measured in approximately 24 individuals from all inbred protein strains at cycles ~65 and ~100 of the selection experiment and are reported in Figure 3.4. The eight possible protein strain-cycle combinations (4 IPS genotypes x 2 cycles) are ordered in rows according to protein concentration, where IHP cycle 100 represents the strain-cycle combination with the highest protein concentration and ILP cycle 95 the lowest. Each column represents a different haplotype or allele with the total number of columns corresponding to the total number of haplotypes or alleles identified in all strain-cycle combinations. Haplotypes or alleles overrepresented in high protein strains are indicated by a white-to-

blue continuum, and alleles overrepresented in low protein strains are indicated by a white-to-red continuum. Boxes without values indicate that of the 24 individuals surveyed, none contained that particular allele for the given cycle-strain combination.

Six major 22-kD α-zein haplotypes (A, B, C, E, F and G) were discovered. Haplotypes B and E were combined into a single haplotype, B&E. Interestingly, the haplotypes demonstrated clear shifts in frequencies that were consistent with protein concentration. For example, the C haplotype was present at a 75% frequency at ILP cycle 65, but decreased to 22% by cycle 95. During this time, the B haplotype increased from 25% to 78%, illustrating a dramatic shift from the B&E to the C haplotype. Frequency shifts were also observed in IHP. For example, the F haplotype decreased from 65% to 45% between cycles 65 and 100. Conversely, the A haplotype increased from 35% to 55%. Furthermore, the two haplotypes identified in ILP (B&E and C) were not detected with any frequency in the individuals sampled from IHP at either cycle-strain combination. Likewise, the two haplotypes in IHP (A and F) were not detected in ILP samples. Clear frequency shifts were evident for the reverse strains, although IRHP seems to have preferentially accumulated a haplotype not observed in any of the strains (haplotype G) with an increasing trend towards the same haplotype increasing in IHP (haplotype A). IRLP is trending towards the same haplotypes as ILP and away from the otherwise IHP-exclusive haplotype F.

Three *prolamin-box factor 1 (*umc1065) alleles were identified with 11, 16, or 17 Asn repeats (rt). The high protein strain-cycle combinations exhibited strong preferential accumulation of the 16-rt allele with complete fixation in IHP65 and IHP100 and near fixation in IRLP100 and IRLP69. The low protein strain-cycle combinations

mostly demonstrated favorable accumulation of the 11-rt allele with the highest frequency observed in ILP95, followed by ILP65 and IRHP67. Although not as strongly fixed for the 11-rt allele as the high protein combinations were of the alternate allele, favorable allele frequency increased between ILP65 and ILP100, indicating a shift towards fixation of the 11-rt allele, at least with respect to ILP. IRHP seems to be an anomaly, where the 11-rt allele was most abundant in cycle 67 but shifted to a significantly higher abundance of the 16-rt allele by cycle 100.

Three *opaque2* (umc1066) alleles with 3, 4 or 5 pro-gln dipeptide repeats were identified from the the previous results section that described cloning and sequencing *o2* in IHP1 and ILP1. The six cycle-strain combinations with the lowest protein concentration were nearly fixed for the 4-rt allele, while IHP was the only strain that exhibited significant heterogeneity; at cycle 65 IHP contained a 71% 4-rt allele frequency, but this decreased dramatically to only 20% by cycle 100 with a subsequent increase in 3-rt allele frequency from 17% to 80%. Thus, IHP is accumulating a different allele than any of the other strain-cycle combinations, with this 3-rt allele only appearing at a very low frequency in only one other cycle-strain combination.

Two *asparaginase* (ASNase) alleles were identified that showed nearly complete divergence between high and low protein strains. The four lowest protein cycle-strain combinations were fixed or nearly fixed for one allele (ASNase-ILP). The four highest protein cycle-strain combinations were fixed or nearly fixed for the other allele (ASNase-IHP). The presence of a LP allele in IHP100, but not in IHP65 indicates that this allele was present in the population at cycle 65, but likely at a very low frequency and thereby not sampled in the 24 individuals surveyed.

Two *asparagine synthetase3* (*AsnS3*) alleles were identified. The two IHP cycle-strain combinations were fixed for the HP allele (*AsnS3*-IHP). The four intermediate protein strain-cycle combinations, including IRHP100, IRLP69, IRHP67 and IRHP100, were fixed for the LP allele. The two ILP strain-cycle combinations exhibited near fixation for the LP allele with increasing frequency between cycle 65 and 95.

Phenotypic Analysis of the Illinois Protein Strain Recombinant Inbreds

The IPSRIs were grown in 2008, 2009, 2011 and 2012. In 2008 and 2009, approximately five IPSRI plants were self-pollinated per genotype, and bulk phenotypic measurements were collected. 2008 and 2009 data had been generated previously using N combustion, and this method only estimates N concentration. In order to introduce the mRFP1 transgene onto the IPSRIs, up to five IPSRI ears were cross-pollinated with B73:*Floury2*-mRFP1 pollen in 2011 and 2012. Individual phenotypic measurements were collected using NIR, which measures the percentages of protein (N), starch, and oil, and Axiovision, which measures the Red/ Green phenotype. Histograms of each trait are shown in Figure 3.5. All traits were normally distributed. Mean protein in the IPSRIs was about 15% and ranged from 8-22%. 2012 exhibited a significantly higher mean protein of 18% and ranged from 11-25%. Neither starch or oil concentration measurements were available from 2008 and 2009, but mean starch was 63% in 2011 with a range from 58-70%. 2012 exhibited a lower mean of 55% with a range from 47-65%. Oil ranged from only a fraction of a percent to over 5% in both 2011 and 2012 with means of 3.3% and 1.9%, respectively. Red/ Green phenotypic means were 1.9 and 2.2 and ranged from 1.2 to 3.6 in 2011 and from 1.2 to 5.0 in 2012.

The relative contribution of variance components, including genotype, year, ear replication within a genotype plot (earrep), and the genotype by year interaction, were determined relative to the total variance of each trait studied, and the results are presented in Table 3.1. Significant genotype, year, and genotype x year effects were observed for all traits, possibly indicating the need to analyze each year separately in subsequent QTL mapping analyses. Because the ear replication term was not significant for protein concentration or the Red/ Green trait, phenotypic means of all ear replications were calculated for each genotype, and the mean phenotypes were used. Although the ear replication term was significant for starch, mean phenotypes were also calculated for this trait to be consistent, methododically. The ANOVA data was used to calculate broad sense heritabilities for each trait. Heritability for protein and starch were 0.55 and 0.53, respectively, but much lower for oil at 0.2. Heritability for the Axiovision Red/ Green trait was higher than any NIR trait at 0.63.

Pearson's correlation analysis was conducted on kernel composition traits in the IPSRI population grown in 2008, 2009, 2011 and 2012. Correlation coefficients and significance levels are presented in Table 3.2. Traits included protein, starch and oil concentration, as measured by N combustion or NIR, and the *Floury2*-mRFP1 Red/Green (R/G) phenotype. From this analysis, strong correlations were observed among traits across years. For example, among the four years for which protein concentration data was available, correlations ranged from 0.48 to 0.65. Relatively strong correlations were observed between 2011 and 2012 for starch (0.6) and Red/ Green (0.67). The only trait that did not exhibit a strong correlation across years was oil, with a correlation of only 0.14 between 2011 and 2012. Strong correlations were

also observed between traits. The highest correlations were observed between starch and protein, which exhibited negative correlations that ranged from -0.48 to -0.91. The highest correlations were observed between starch and protein *within* the same year, which were -0.91 (2011) and -0.83 (2012). Correlations between oil and either starch or protein were insignificant. Significant positive correlations were observed between protein concentration and the *Floury2*-mRFP1 Red/Green (R/G) phenotype in 2011 (0.48) and 2012 (0.33).

Due to the large variation in flowering time observed previously among the IPSRIs, IPSRI plants were phenotyped for the number of days it took for approximately half of the plants within a row to reach anthesis. This phenotype is referred to as "days to flower" and was included to help control variation in protein or starch concentration that may potentially arise due to variation in period of vegetative growth. The days to flower phenotype exhibited a normal distribution that ranged from 59 to 87 days, excluding two outliers, 46 and 96 days. Mean and mode days to flower were 72 and 68 days, respectively. Finally, juvenile leaf wax was scored according to presence (wild-type) or absence (*glossy15* mutant). Seven out of 138 IPSRI genotypes were scored as *glossy15* mutants.

Candidate Gene-Trait Associations and Genome-wide SNP-Trait Associations

A number of molecular markers for candidate genes in both the Asn-cycling and zein synthesis pathways were associated with IPSRI mean kernel composition phenotypes. These markers were manually included with the 70K SNPs used for genome-wide SNP-trait analyses. The results of candidate gene-phenotype

associations will be discussed first. Candidate genes in the Asn-cycling pathway and their respective molecular markers included: *asparaginase* (ASNase and ASNase3UTR), *asparagine synthetase3* (*AsnS3*), and the putative light-regulated ortholog of *Arabidopsis bZIP1* that is hypothesized to regulate maize *asparagine synthetase3,* GRMZM2G024851 (bZIP). Candidate genes in the zein synthesis pathway and their respective molecular markers included: *opaque2* (umc1066), *prolamin-box factor1* (umc1065), two SSR markers in the z1A cluster (umc1943 and umc2150), and one SSR marker in the z1C cluster (p-bnlg421). In addition to candidate genes, approximately 70K (69,515) out of the 955K unimputed SNPs called in the E. Buckler production pipeline (July 2013 build) (Glaubitz et al, 2014) contained 90% or less missing data in the IPSRIs and were bi-allelic. This 70K SNP dataset was used in subsequent SNP-trait associations.

Negative $\log_{10}$ (pvalues) of associations with protein and starch concentration, and the Axiovision Red/ Green phenotype, are plotted in Manhattan plots in Figure 3.6. Associations with juvenile leaf wax are plotted in Figure 3.7. The most significant candidate genes are indicated in the Manhattan plots. Negative $\log_{10}$ (pvalues) of candidate genes are also reported in Table 3.3. Of all candidate genes, *AsnS3* exhibited the strongest associations with both protein (Figure 3.6A) and starch concentration (Figure 3.6B). *AsnS3* negative $\log_{10}$ (pvalues) for protein and starch were 2.61 and 2.27, respectively. The second strongest association was observed between GRMZM2G024851 (*bZIP)* and starch, with a negative $\log_{10}$ (pvalue) of 2.10. *ASNase* exhibited the strongest association with Red/ Green (Figure 3.6C) with a negative $\log_{10}$

(pvalue) of 2.52. No candidate genes were strongly associated with juvenile leaf wax. The highest negative $\log_{10}$ (pvalue) was 0.79 (p-bnlg421).

The list of significant SNPs for each trait was queried against the B73 reference genome (version 2) (Schnable et al., 2009) to generate a list of GRMs whose basepair coordinates were within 10kB either upstream or downstream. The GRMs were then blasted against the MaizeGDB database (Lawrence et al., 2004) to retrieve annotation information associated with GRMs. If maize annotations were not available then orthologous annotations for either Oryza *sativa* (rice) or *Arabidopsis thaliana* were included. *O*rthologous annotations are preceded by "Os:" or "At:" to indicate that this they are not the true maize annotations. This list was generated in part by Jennifer Arp of the Moose lab. Traits included in this analysis were protein and starch concentration, Red/ Green, and juvenile leaf wax. The query was performed using the top 70 associations out of the approximately 70K SNPs, which is equivalent to a false discovery rate of (α=0.001). The list of significant SNP-trait associations and GRMs can be found in Table 3.4. Multiple entries for the same SNP (BPPos) may occur due to the presence of multiple GRMs within the 20kB segment surrounding the SNP. SNPs for which no GRM was nearby (within 10Kb) are listed in supplementary Table 1.

Of the GRMs found to be within 10Kb of a significant SNP (FDR, α=0.001), 29 unique GRMs were identified for protein, 29 for starch, 32 for Axiovision Red/ Green, and 19 for juvenile leaf wax (Table 3.4). Eight GRMs were commonly identified for protein and starch on chromosome 2 (GRMZM2G361388), chromosome 3 (GRMZM2G039867 & GRMZM2G062524), chromosome 7 (GRMZM2G041163, GRMZM2G041175 and GRMZM2G339728), and chromosome 8 (GRMZM2G516301 &

GRMZM2G084489). GRMs that were common to protein and starch are highlighted. Interestingly, no significant SNPs for the Axiovision Red/ Green trait overlapped with those identified for protein or starch.

For genome-wide SNP-trait associations, negative $\log_{10}$ (pvalues) ranged from 3.97 to 6.07 for protein. Several of the most significant SNP associations with protein co-localize to a few regions of chromosome 1 at approximately 45Mb and between 56Mb and 61Mb. These two regions can easily be visualized as two distinct peaks that surround *AsnS3* in Figure 3.6A. For starch, negative $\log_{10}$ (pvalues) ranged from 3.12 to 5.2. For the Axiovision Red/ Green phenotype, negative $\log_{10}$ (pvalues) were higher than NIR tratis and ranged from 3.34 to 10.32. For juvenile leaf wax, negative $\log_{10}$ (pvalues) were higher than any other trait and ranged from 4.73 to 15.16.

Interestingly, out of the 70 most significant SNP-trait associations with juvenile leaf wax, 64 SNPs localized to the chromosome 9, which is the same chromosome to which *Glossy15* has been genetically mapped to (Table 3.4 and Supplementary Table 3.1). In this list of 70 most significant SNPs, the SNP in closest proximity to the *Glossy15* locus (bp coordinates 95,739,338- 95,742,689, B73ref V2) was the SNP at 100,992,537 bp, over 5Mb downstream (Table 3.4). The negative $\log_{10}$ (pvalue) of this SNP was 6.96. Surprisingly, thirty-one SNPs were more significant than this SNP, and their negative $\log_{10}$ (pvalues) ranged from 7 to 16. However, the closest SNP was positioned around 73Mb, 20Mb away from the *Glossy15* locus. Furthermore, three other SNPs in the 70K SNP dataset were located within the *Glossy15* gene, but were not among the 70 most significant SNPs (Figure 3.7). The coordinates and negative

$\log_{10}$ (pvalues) of these SNPs are 95,739,846 (3.53), 95,739,880 (3.77), and 95,739,930 (3.69).

Because the most significant associations with juvenile leaf wax were not SNPs within *Glossy15*, as expected, it was hypothesized that the sequencing reads could have been aligned to the wrong physical location by the pipeline (Glaubitz et al., 2014). This would have resulted in incorrect basepair positions for SNPs within this region. To test this hypothesis, two additional molecular markers found in the promoter (umc1681) and 3'UTR (umc1691) of *Glossy15* were genotyped in the IPSRI mapping population, and their haplotypes were compared to 161 of the most significant SNPs on chromosome 9, including the three SNPs within *Glossy15*. It was predicted that if their haplotypes were similar to the SNPs 20Mb away then this could indicate a mis-alignment of the reads. However, this was not the case. The two umc markers co-segregated, as expected, but their haplotypes closely followed those of the three SNPs within G*lossy15*. This haplotype extended upstream as well to SNPs up to 4.5Mb away. None of the GRM annotations for the most significant SNPs (eg. SNPs 20Mb upstream) for juvenile leaf wax offered significant clues as to a relevant function (Table 3.4).

Linkage Map Construction

The 70K SNP dataset was filtered to develop a high confidence SNP dataset for genetic map construction using three main criteria. The criteria were as follows: 1.) the minor allele frequency (MAF) was greater than 0.43 and less than or equal to 0.5, 2.) the SNPs weregenotyped in 90% or greater individuals, and 3.) the SNPe were fixed for different alleles between IHP1 and ILP1. 733 identical loci were also removed, resulting

in 4,118 SNP markers. IPSRI individuals were also filtered for percentage of missing data, where individuals genotyped for less than 50% of loci were discarded. This resulted in the removal of IPSRI 77 and 100. IPSRIs 436, 289, and 214 were also discarded due to higher than expected heterozygosity compared to the other IPSRIs, which might have resulted from contamination during sampling or DNA preparation.

Marker grouping and linkage map construction were performed using the maximum likelihood method (independence LOD = 6.0) in Joinmap 4.1 software. A total of 67 groups containing five or more SNPs were manually ordered according to chromosome and SNP basepair positions. Groups that displayed locally large gaps in map distance that corresponded with non-sequential basepair SNP coordinates on either side of the gap were also manually broken and re-ordered within the correct linkage group according to SNP basepair positions. Then to stitch together the linkage groups within a chromosome, the map distances across the gaps were estimated by dividing the difference in basepairs of markers on either side of the gap by the average physical distance per map unit for that chromosome. The number of genetic markers, map length and average number of basepairs per cM unit per chromosome are reported in Table 3.5. Genome-wide, the average number of basepairs per map unit was 439,369. The total number of markers was 3,679, including candidate gene markers, and the total genetic map length for all ten chromosomes was 6,258.73. The assembled linkage map is reported in Figure 3.8 and Supplementary Table 3.2.

Discussion

One primary goal of Quality Protein Maize breeding programs is to reduce α-zein protein accumulation because it is deficient in several essential amino acids. However, with the exception of known bZIP transcriptional activator *Opaque2* (Schmidt et al., 1992) and putative Dof class transcriptional regulator *Prolamin-box factor1 (PBF)* (Vicente-Carbajosa et al., 1997; Marzabal et al., 2008), α-zein regulation remains poorly understood. The genetic populations and lines derived from the Illinois selection experiment offer unique advantages for studying kernel composition traits because divergent recurrent selection in this experiment has specifically altered α-zein protein accumulation. This observation was reported previously for cycle 90 inbred Illinois Protein Strains (Bhattramakki, Sachs and Kriz, 1996), and confirmed here by SDS-PAGE analysis comparing IPS cycles 65 and 105. From this experiment, large differences in 19-kD and 22-kD α-zein protein were observed between cycles 65 and 105 that were consistent with the direction of selection in each Illinois Protein Strain. For this reason, the genetic resources derived from the selection eperiment were utilized in a series of experiments aimed at understanding the genetic regulation of α-zein gene expression. These included a combination of expression and allele profiling experiments, and genetic association studies.

The lack of evidence for dosage effect or maternal imprinting as potential mechanisms of the maternal inheritance of grain protein, as tested in chapter two, implicates plant nutrient status as the most likely mechanism, although differential methylation may also be important. The nutrient status hypotheis is supported by hyperaccumulation of Asn in IHP and the concurrent activities of enzymes involved in

Asn synthesis (Lohaus et al., 1998; Church, 2008), which essentially regulate plant nutrient status by either directing N for storage in the form of Asn or converting it to molecules more suitable for growth, including Gln and Asp. Patterns of Gln and Asn synthesis in hybrids following pollination are consistent with this finding, where drastic decreases in Gln were countered by simultaneous increases in Asn (Seebauer et al., 2004). Zein transcriptional regulation is also thought to be important, and it is plausible that transcriptional activation or duration of storage products may only occur in response to upstream signals, possibly conferred by Asn levels or the ratio of Gln: Asn (Seebauer et al., 2004). The Asn-cycling pathway is largely responsible for the relative abundances of these amino acids, and the genes involved in Asn-cycling are thus key candidate regulators of plant nutrient status. As such, they were hypothesized to have been important targets of selection for kernel composition traits, along with genes directly involved in transcriptional regulation of the α-zeins.

To test the role of candidate genes in the Asn-cycling and zein synthesis pathways as potential targets of selection in the Illinois selection experiment, three main studies were conducted. First, the availability of cycles 65 and 100 IPS populations permitted comparisons of allele frequencies over a forty year interval. The profiling results demonstrated the presence of major zein haplotypes and clear deviations from HWE. Candidate genes exhibited fixation or near fixation of alternate alleles that were consistent with protein concentrations of the respective strains. An interesting exception to predicted patterns was observed for the IRHP strain. While all other low protein cycle-strain combinations preferentially accumulated the 11-rt allele of *PBF*, IRHP retained the allele common to high protein cycle-strain combinations, the 16-rt

allele. This may indicate that the 11-rt allele is not necessary to confer the low protein phenotype of the IRHP strain and that IRHP achieves a low protein phenotype through mechanisms that are distinct from those of ILP. The sharp decrease in protein of IRHP in the first fifteen cycles of reverse selection, which represents the fastest rate of gain of any cycle over the course of the Illinois selection experiment (Dudley, et al., 2004) supports this theory (Lucas et al., 2013). Thus, while *PBF* might be an important factor of the other strains, at least according to allele frequencies, its role for IRHP is unclear. Additionally, two rare alleles were identified, including the *PBF* 17-rt allele in IRHP and the 5-rt *O2* allele in IHP. However, because their frequencies did not shift dramatically between cycles 65 and 100, it is highly unlikely that these alleles were important for achieving favorable phenotypes in either strain. The presence of strain-specific alleles could have resulted from spontaneous mutations, or may indicate a founder effect when the populations were established. It is also possible that rare alleles were present in the other strains, but that they were not sampled in the 24 individuals assayed. Overall, deviations from HWE suggest that these candidate genes were targeted by selection.

Secondly, to document α-zein mRNA abundance, expression profiling studies were conducted on the inbreds and the cycles (65 & 105). Expression profiling of IPS inbreds (16 DAP seeds) revealed large differences in mRNA of 19- and 22-kD α-zein genes and transcriptional regulators, *O2* and *PBF,* that were consistent with known levels of α-zein and total protein concentration. This finding establishes a strong connection between mRNA abundance and protein accumulation and indicates that regulation of α-zein gene expression occurs, at least partially, at the level of transcription. Furthermore, the reverse selection experiments demonstrate that these

regulatory changes retained plasticity, as is exemplified by intermediate levels of a-zein, *O2,* and *PBF* expression in IRHP and IRLP.

While expressional profiling of the inbred IPS reduces the complexity associated with segregating populations, the inbreds provide only a snapshot of mRNA variation at cycle 90 of the selection experiment. To investigate dynamic changes over time, cycles 65 and 105 were also assayed. Significant differences in gene expression consistent with protein concentration were observed by cycle 65 for both 19- and 22-kD α-zein genes, which is consistent with expression patterns of the inbred IPS. However, the relative expression of *O2* and *PBF* genes did not appear to be associated with dramatic responses to selection prior to cycle 65; divergent expression was only observed by cycle 100. This observation is interesting considering the observation of fixed or nearly fixed DNA variants by cycle 65 (IHP65 for *PBF* and ILP65 for *O2*). It is possible that these candidates were subject to genetic drift. Illinois High Oil and Illinois Low Oil strains could be genotyped to estimate allele frequencies of unselected populations derived from the same founders. The lack of evidence for divergent expression of *O2* or *PBF* prior to cycle 65 suggests the possibility for other important regulatory factors in the early selection cycles. Expression profiling of VT leaves revealed up-regulation of *AsnS3* and *bZIP* and down-regulation of *ASNase* in IHP, and the reverse for ILP. These patterns are consistent with protein accumulation and corroborated by near fixation or fixation of DNA variants by cycle 65. Based on these results, it appears that Asn-cycling genes were targeted in earlier cycles of the Illinois Selection Experiment.

The third experiment for testing if candidate genes were targeted by selection involved gene-phenotype associations with the IPSRI mapping population. Unlike with

allele frequency or expression profiling, which essentially test for correlation and can be subject to the effects of genetic drift, this experiment allowed for empirical testing of the genetic effects of candidate genes. IPSRI phenotypes included protein and starch concentrations and the Axiovision Red/ Green phenotype. While oil concentration data was collected on the IPSRIs, the heritability of this trait was very low, which may be attributed to the pollen effect on kernel oil concentration (Letchworth and Lambert, 1998) as a result of necessarily having to cross B73:*floury2*-mRFP1 to the IPSRIs. Perhaps a higher heritability would be observed if the IPSRIs were self-pollinated, although because the IPSRIs segregate for protein and starch, they do not represent the best choice for investigating oil concentration. For this reason, oil was not included in subsequent analyses. It is also important to note that because the IPSRIs were derived from cycle 70 of the selection experiment, these tests can only provide information about which loci were important before or around cycle 70.

To gauge background significance level, genome-wide negative control SNP markers were developed using genotype-by-sequencing and included in the candidate gene-phenotype association studies. While no candidate genes were identified in the list of 70 most significant SNPs according to an FDR ($\alpha = 0.001$), this result is a symptom of the large number of independent tests and the stringency of multiple testing correction procedures, such as FDR or Bonferroni. Alternatively, significance thresholds can be determined biologically, based on the significance levels of candidate genes. This would increase alpha, and more SNPs would be declared significant, but it should be noted that this could lead to an increase in false positives as well. Here, it was appropriate to use both statistically and biologically derived thresholds. Out of the

ten candidate genes tested, the gene that most strongly associated with protein and starch was *AsnS3.* The putative *bZIP,* also exhibited a strong association with starch concentration. The gene that most strongly associated with the Red/ Green phenotype was *ASNase*. The detection of Asn-cycling pathway genes in the IPSRIs is consistent with divergent expression and allele frequencies by cycle 65.

It is interesting that NIR phenotypes detected *AsnS3,* while the Red/ Green phenotype detected *ASNase.* Perhaps this is an artifact of how each phenotype is measured. Because NIR forces components to sum to 100 percent, it may artificially inflate the inverse relationship between protein (N) and starch (C). This difference may be more readily detected by *AsnS3* because of its direct influence on the C:N balance in the plant and seed. Additionally, because NIR measures amine bonds, it cannot separate between proteins and free amino acids. Conversely, the Red/ Green phenotype specifically tracks α-zein protein concentration and gene expression, so it should more readily detect factors closely associated with α-zein regulation. It is possible that the increased sensitivity to degradation of Asn by the enzyme ASNase results from its putative function as a signal of vegetative N-assimilates available for storage protein synthesis.

O2 and *PBF* did not significantly associate with any trait. This can be explained by the fact that these genes did not exhibit differential expression until cycle 100 and thus would not be detected by a population (IPSRIs) derived from cycle 70. This finding is also consistent with a lack of detection in prior QTL mapping studies using the RM7 IHP x ILP population even though it was segregating in the IHP and ILP strains at cycle 65. It is also possible that *Opaque2* is not detected because the allelic variation simply

may not lead to strong enough phenotypic effects. This is supported by the finding that the recessive *o2* null mutation only reduced protein by 1% when introgressed into the ILP1 inbred and only 4% when introgressed into the IHP1 inbred (Lucas et al., 2013). Because ILP1 protein concentration is closer to normal maize varieites, the average reduction in protein by *o2* in normal maize is likely around 1%, which is likely the *maximum* phenotypic effect and could explain why it is not detected in other mapping populations. That *O2* was never detected may also indicate a lack of sensitivity with respect to the phenotypic data collection method, NIR. It also suggests the possibility for additional unknown genetic factors acting either within the zein pathway or upstream, or both.

Interestingly, no candidate molecular markers within the 19-kD or 22-kD α-zein clusters significantly associated with IPSRI phenotypes even though they did exhibit large expression variation by cycle 65. This result was also reported by Cook et al. (2004) and suggests that *trans-acting* regulatory variation may be more important than *cis-acting* variation for altering α-zein gene expression. This is also supported by the finding in chapter two that documented approximately equivalent *Floury2*-mRFP1expression among three different transgenic events because it indicates that α-zein gene regulation is not dependent on chromosomal location. Also consistent with this hypothesis is the observation of coordinated regulation of all 19- and 22-kD α-zein genes. Because α-zein proteins can be encoded by more than 50 possible genes, simultaneous changes in DNA, related *cis*-acting elements or coding regions of these genes might not be the most efficient biological approach for altering phenotypic variation. Alternatively, adjusting the expression of common transcription factor(s) is

expected to coordinately regulate a large number of genes efficiently, and they appear to be more commonly associated with major quantitative trait loci (QTL) for complex traits (Fong, Joyce and Palsson 2005, Swanson-Wagner et al. 2006). Asn-cycling pathway candidates acting in *trans* might serve this function by altering Gln: Asn ratios in vegetative tissues, which then presumably serve as downstream signals for storage product synthesis. Fine-tuning of zein expression may then occur by factors that regulate subsets of α-zein genes, such as *O2*, or through other *cis-acting* variation, such as promoter mutations. Differential localized methylation may also achieve this result.

While a significant association was observed between *bZIP* and starch concentration, the primers could not distinguish between three putative gene copies. However, gene expression data of potential candidates could be exploited to narrow down the possibilities. Because only one EST has ever been associated with GRMZM2G116494 (chr 6), this copy is likely a pseudogene. Of the other two copies on chromosome 3, it cannot be determined whether the positive association was with GRMZM2G02485 or GRMZM2G143290. It is likely that qRT-PCR primers were also unable to uniquely report expression of a single copy, but rather a summation of expression of both copies. A recent RNASeq study by another lab member confirmed differential expression of GRMZM2G024851 between IHP and ILP, which lends support to the role of this gene copy. This study also identified a fourth candidate annotated as a bZIP protein (GRMZM5G858197), also on chromosome 3. The expression pattern of this candidate closely mirrored *AsnS3* in the seed, peaking at 8 DAP and down-regulated in ILP1 compared to IHP1 or B73. However, the negative $\log_{10}$(pvalues) of the SNPs (BPPos 175,551,452 and 175,700,060) closest to GRMZM5G858197 were

insignificant. The RNASeq reads in this other study were able to uniquely align reads to gene copies GRMZM2G02485 and GRMZM2G116494, and possibly also GRMZM2G143290, so this data could be mined for polymorphisms between IHP and ILP in order to develop primers specific to each gene copy. As of now, GRMZM2G143290, GRMZM2G024851, and GRMZM5G858197 all represent candidate orthologs of the *At. bZIP1* gene that is hypothesized to regulate *AsnS3.*

In addition to providing a background significance threshold for candidate gene-phenotype associations, GBS data collected on the IPSRIs permitted genome-wide SNP-triat associations. The advantages of using a GBS approach for development of SNP markers are that it is relatively inexpensive, generates hundreds of thousands of markers, and that it overcomes ascertainment bias of array based approaches. However, one major disadvantage is that it generates large amounts of missing data (40-80%). This poses several challenges. For example, low read depth can make it difficult to accurately identify heterozygous loci in highly heterozygous unrelated individuals. Fortunately, the IPSRI mapping population has undergone 6 generations of inbreeding, and the inbreeding coefficient (1- 0.5^6 = 0.984375) is expected to be very high and the frequency of heterozygous loci very low. The number of loci genotyped [in all individuals] can also be problematic. To overcome this limitation, a number of imputation methods have been developed for various population types, including bi-parental RIL populations. However, while the IPSRI mapping population is the closest in structure to a bi-parental RIL mapping population, there are a few caveats that warrant using unimputed data only. First, the IPSRIs were not generated by a strict bi-parental cross, but rather by the cross of 5-7 IHP individuals with 5-7 ILP individuals,

meaning that up to 14 different alleles may have been transmitted to the RIL progeny. Secondly, the parental genotypes were not saved when the population was initiated, making it difficult to determine which [of the 14 or fewer] alleles were transmitted from IHP versus ILP individuals. To overcome these complications, a number of marker filtering criteria were adopted for the different experiments conducted here.

For the genome-wide SNP-trait associations, the most important criteria was that the SNPs were bi-allelic in order to reduce complexity associated with multiple parental alleles. The markers also had to contain 90% or less missing data. Approximately 70K SNPs met these criteria and were subsequently associated with NIR phenotypes. Using an FDR cutoff (α=0.001), 70 SNPs were declared significant for each trait. When SNP basepair coordinates were queried against maize GRM v2 coordinates, 29 unique GRMs were identified for each protein and starch and 32 for the Red/ Green phenotype. Additionally, eight GRMs were significantly associated with both protein and starch. This finding is not surprising given the high degree of pleiotropy that has been reported in previous QTL mapping studies for kernel composition traits, where many QTL were commonly identified for both protein and starch (Goldman et al., 1993; Dijkhuizen et al., 1998; Sene et al., 2004; Dudley et al., 2004; Dudley et al., 2007; Cook et al., 2012). Furthermore, the discovery of common GRMs actually validates the statistical robustness of this analytic method. That no SNPs were commonly detected between Red/ Green and NIR might be expected given that each method detects different biological processes. The only moderate positive correlation between the Red/ Green phenotype and protein concentration (NIR) supports this.

The list of annotations was obtained from MaizeGDB based on the 181 release of Phytozome v7.0 that includes 5b.60 annotations (Schnable et al., 2009), where informative maize annotations were maintained if possible. Otherwise, annotations for orthologous genes in either *Oryza sativa* or *Arabidopsis thaliana* were included based on phytozome annotations. Follow-up of the GRMs common to multiple traits is recommended first. In addition, more confidence should be given to GRMs associated with multiple, clustered significant SNPs, and spurious single SNPs should be regarded with caution. Transcription factors are strong candidates because they are *trans-acting* regulatory variants that can efficiently alter gene expression of numerous gene copies in a coordinated fashion, as was observed with the zeins. The A.*thaliana* ortholog of one GRM commonly identified for protein and starch was annotated as a WRKY DNA-binding protein. Several GRMs identified by associations with the Red/Green phenotype were also annotated as transcription factors, including factors belonging to the WRKY superfamily and genes with bZIP, myb, and zinc finger domains. If these annotations are true indicators of gene function then this would confirm an increased ability for the Red/ Green phenotype to more readily to detect regulatory variants due to the fact that it tracks expression variation.

The Red/ Green phenotype also detected a GRM on chromosome 10 whose *A.thaliana* ortholog (AT1G12110.1) was annotated as a nitrate transporter, NRT1.1 (CHL1). Expressed in lateral roots, NRT1.1 is thought to act in external nitrate sensing to enable localized exploitation of nitrate through lateral root proliferation. Compared to wild-type plants, mutant *chl1*plants exhibited significant reductions in lateral root growth that ultimately led to biomass reduction (Remans et al., 2006). Additionally, a lack of

NRT1.1-mediated uptake has been hypothesized to up-regulate NRT2.1, which plays an active role in N-uptake (Munos et al., 2004). A recent study examining root morphology of IHP and ILP plants found significantly more lateral root development in IHP consistent with N scavenging (Topp et al., 2013). IHP also exhibits a greater N-uptake efficiency compared to ILP (Below et al., 2004). Therefore, it is easy to envisage how differential activity of an orthologous NRT1.1 gene might influence these morphological and physiological differences. Massive parallel signature sequencing (MPSS) data previously generated on IHP and ILP root tissue samples lends support to the importance of this locus as well. IHP roots exhibited 2- to 3-fold more transcripts of the signature sequence (GATCGTCGTCGGTCAGA) that maps within 40Kb of the SNP identified here on chromosome 10.

Due to the segregation of an apparent *glossy15* mutant phenotype in the IPSRIs, juvenile leaf wax phenotypes were collected on the IPSRIs as a positive control in SNP-trait association studies. It was hypothesized that if the *glossy15* mutation was the cause of the characteristic glossy leaf phenotype in the IPSRIs, then SNPs within or linked to *Glossy15* on chromosome 9 would significantly associate with corresponding IPSRI phenotypes. A cluster of SNPs on chromosome 9 significantly associated with the phenotype, but the most significant SNPs were 20Mb away from the *Glossy15* locus. Three SNPs identified within the *Glossy15* gene itself were not significantly associated, at least according to an FDR (α=0.001), although they were more significant than the majority of SNPs, genome-wide. To investigate whether SNP basepair coordinates could be incorrect due to potential misalignment of sequencing reads, the IPSRIs were genotyped for known variants in either the *Glossy15* promoter (SSR) or

3’UTR indel, and their haplotypes were compared to haplotypes of 161 of most significant SNPs on chromosome 9, including SNPs within and around *Glossy15*. The haplotypes closely followed those of the SNPs within and around *glossy15* but not those of the other more significant SNPs, which suggests that the reads were aligned to the correct location. Because the SNPs within *Glossy15* were still more significant than the majority of other SNPs, genome-wide, this result can still be viewed as a positive control, although it is suspicious that more significant associations were observed with upstream loci.

Significant associations with SNPs upstream of *Glossy15* may result for two reasons. First, they could represent true associations with additional unknown regulators on chromosome 9. Secondly, they could represent an issue with the B73 reference genome assembly, which would result in incorrect basepair coordinates. One method that would address both possibilities is to genotype markers on both the B73 x Mo17 (IBM) population and the IPSRIs and re-build the genetic map in this region. 20-30 SSR markers spanning upstream of the region containing the most significant associations to downstream of *Glossy15* would likely be sufficient. This would allow for assessment of marker proximity to *Glossy15.* If the markers closest to the most significant SNPs remain distal to *Glossy15* then this would suggest the presence of additional unknown regulators. If their positions are determined to be closer to *Glossy15* then it is likely that there is an error with the reference genome assembly in this region.

Generation of a linkage map was conducted for purposes of QTL mapping. Due to several caveats about the IPSRI mapping population, namely the use of multiple

parents in the original cross of IHP x ILP (cycle 70), sringent filtering criteria were adopted to generate a high confidence SNP data set. Up to fourteen alleles could have been transmitted to the IPSRIs from seven IHP and seven ILP parents. As a result, a proportion of the markers were expected to deviate from the expected segregation of 1:1. Because segregation distortion can lead to false positives in subsequent QTL mapping studies, SNPs deviating from a 1:1 segregation ratio were discarded (0.43<MAF<=0.5). Additionally, only SNPs fixed for different alleles between the IHP1 and ILP1 inbreds were retained. Finally, to further reduce errors associated witih small sample size, SNPs were required to be genoyped in at least 90% of the RILs. Removal of identical loci was performed to minimize the computation time required for ordering markers within linkage groups and because they do not provide further map resolution.

Despite stringent filtering criteria and a stringent independence LOD (6.0) for map ordering, the number of linkage groups generated by JoinMap 4.0 (J.W. Van Ooijen, 2006) was much larger than the expected number, ten. Reducing the stringency led to fewer linkage groups, but then each group contained markers from multiple chromosomes. The large number of linkage groups is likely an artifact of the high degree of random mating in the IPSRI population. Breaks in linkage groups within a chromosome may indicate the presence of recombination hotspots, which would increase genetic map distances to the point where adjacent groups no longer appear linked, regardless of physical distances between markers. Candidate gene markers were included in the marker ordering process, and with a few exceptions, their placement within the linkage groups closely followed their physical basepair coordinates. A few exceptions were the SSR markers within *glossy15,* which were

placed upstream of where their basepair coordinates would suggest they should be. Combined with the previous result of strong associations with SNPs upstream of *glossy15,* this finding may suggest a large inversion on chromosome 9 where the break could have occurred close to the *glossy15* locus. The bZIP marker was also an anomaly, placed on chromosome 6 rather than chromosome 3. This is not surprising given the apparent duplication of at least part of this gene sequence on chromosome 6, as described earlier.

Due to the large number of linkage groups, it was necessary to manually order the groups based on the basepair coordinates of the markers within each group. As a result, genetic map distances between gaps had to be estimated rather than empirically determined. For this reason, any QTL discovered within gaps in subsequent mapping studies should be regarded with caution, where addition of markers within gaps should improve resolution. Overall, due to the large number of markers used to construct the linkage map and the high degree of random mating of the IPSRI population, it is expected that a greater mapping resolution will be achieved in subsequent QTL mapping studies.

The series of experiments conducted here represent an experimental pipeline for validation of additional gene candidates. Candidate genes may first be genotyped in 24 individuals from the cycles (65 & 105) to test for deviations from HWE. Secondly, mRNA expression can be assayed in the inbreds and the cycles (65 & 105) to test for patterns consistent with protein concentration. Finally, candidate genes can be associated with IPSRI phenotypes and/or QTL mapping performed as an empirical test of genetic effect. The merging of these strategies should also supply information about

when a gene candidate was targeted by selection, where allele profiling can distinguish between cycle 65 and 100, and the IPSRIs can verify any candidate that was important until cycle 70.

Conclusion

The roles of candidate genes involved in both zein synthesis and Asn-cycling were tested here using a combination of methods, including expression and allele profiling, and candidate gene-phenotype association studies. The collective results of these studies confirm the roles of both pathways and strongly suggest the importance of Asn-cycling genes in earlier selection cycles and zein transcriptional regulators only after cycle 65. The genetic validation of Asn-cycling genes provides additional evidence that storage protein synthesis may be largely dictated by the availability of N-assimilates in the plant, which is largely dictated by the roles of *AsnS3,* a putative *bZIP,* and *ASNase*. The lack of evidence for dosage effect of *Floury2*-mRFP1 expression and minimal evidence for imprinting, as presented in chapter two, also leave the plant nutrient status hypothesis as the strongest contender.

Eight new candidate genes were commonly identified for protein and starch in the genome-wide SNP-trait association study. This finding is consistent with previous reports of pleiotropy for kernel composition traits and lends confidence to their importance. On the contrary, because the Axiovision Red/ Green phenotype tracks α-zein expression, it detected more GRMs annotated as transcriptional regulatory variants than NIR. It also detected a GRM whose ortholog in *Arabidopsis thaliana* was annotated as a nitrate transporter, which was found to exhibit higher expression in IHP

roots than ILP roots. The validation pipeline presented here, consisting of allele frequency profiling and gene expression analyses, could be employed as first steps for validating additional candidates. The next logical addition to the pipeline is to build a linkage map and conduct QTL mapping as a means of further validation. The full set of 500 IPSRIs could be mined for recombinant plants in order to fine map regions of interest. Additionally, candidates could be cross-referenced to previously identified QTL, such as those reported by Cook et al. (2012), of course acknowledging the possibilitiy for different effects in different germplasm. Finally, functional genetics approaches, such as overexpression or knock-out studies, may be conducted.

Figures

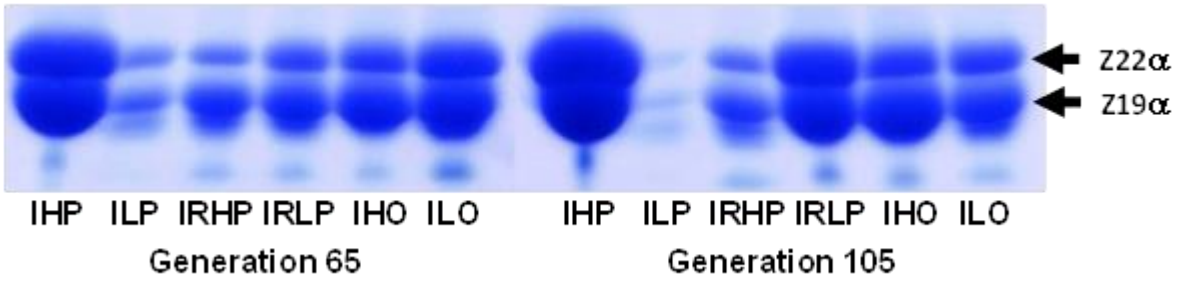
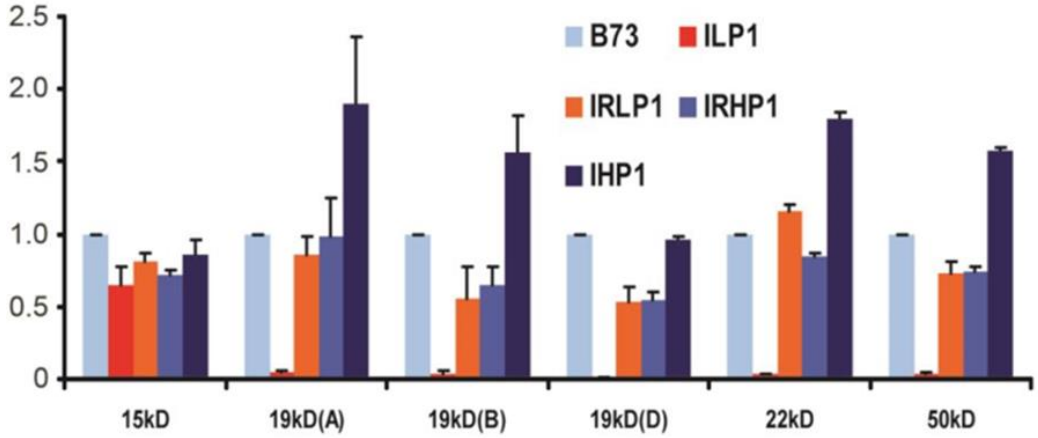


Figure 3.1 SDS-PAGE of alpha-zeins from the Illinois Protein Strains at cycles 65 and 105. Strains included Illinois High Protein (IHP), Illinois Low Protein (ILP), Illinois Reverse High Protein (IRHP) and Illinois Reverse Low Protein (IRLP). Illinois High Oil (IHO) and Illinois Low Oil (ILO) are included as unselected controls. The 22-kiloDalton α-zeins (Z22α) and 19-kiloDalton α -zeins (Z19α) are indicated by arrows.

A.



B.

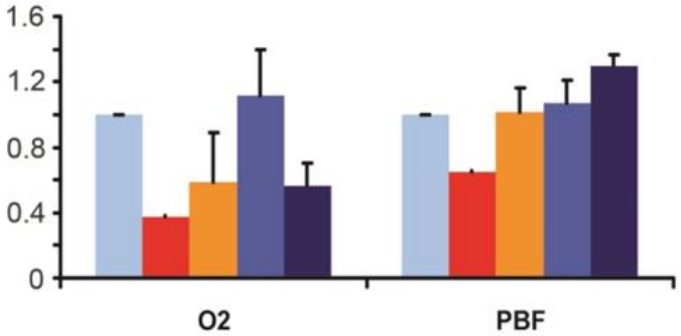


Figure 3.2. Relative mRNA expression of (A) zein pathway genes and (B) zein regulators in the inbred Illinois Protein Strains and inbred control B73 (16 DAP seeds), as determined by qRT-PCR. Strains included Illinois High Protein (IHP), Illinois Low Protein (ILP), Illinois Reverse High Protein (IRHP) and Illinois Reverse Low Protein (IRLP). Z19α subfamilies indicated (A, B and D).

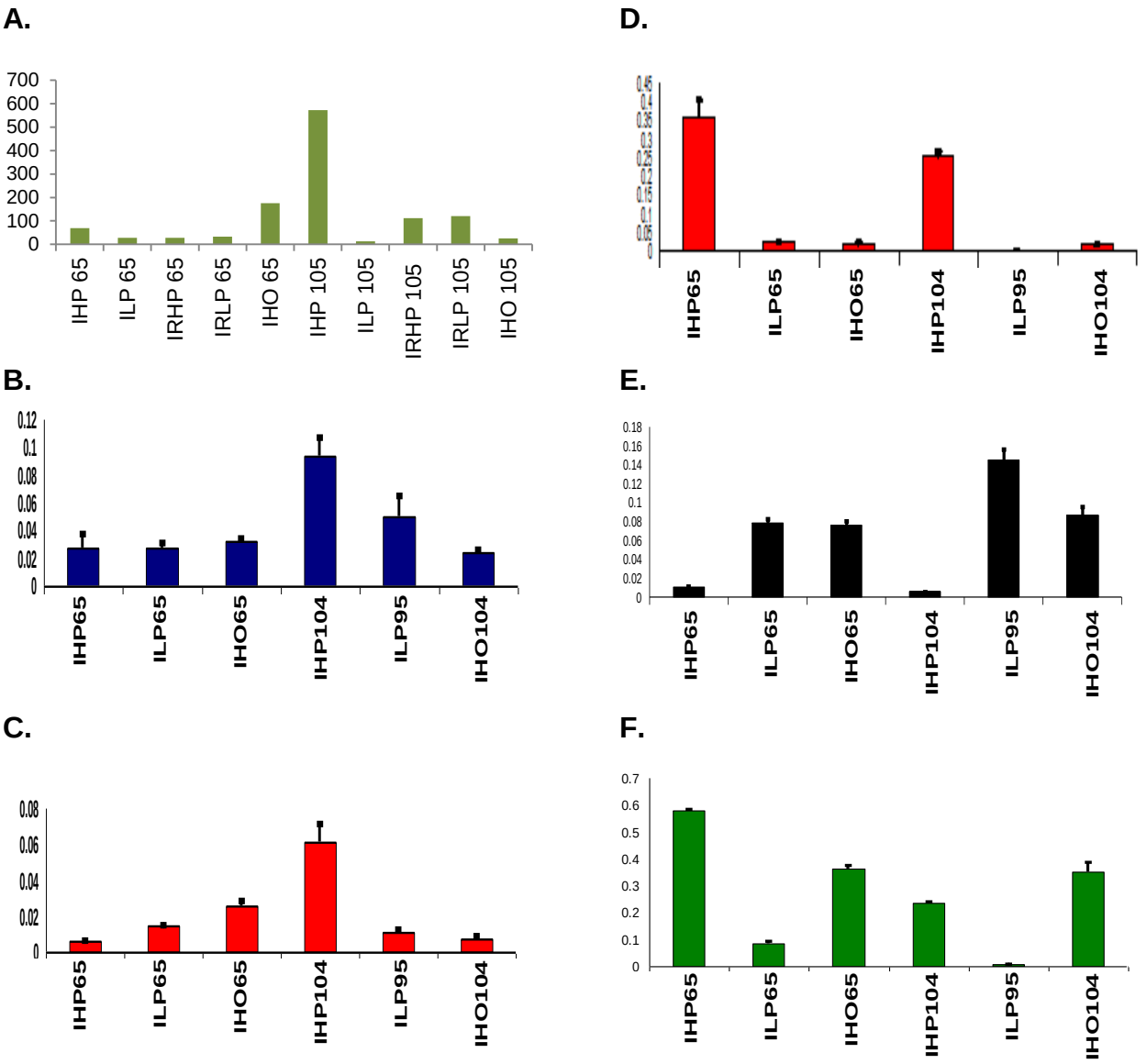


Figure 3.3. Relative mRNA abundance of (A) *Floury2 (Z22α)* (B) *Opaque2 (O2)* and (C) *Prolamin-box factor (PBF)* (D) *Asparagine synthetase 3 (AsnS3)* in 16 DAP seeds, and (E) *asparaginase (ASNase)*, and (F) the putative maize ortholog of the *At*. *bZIP1* in the ear leaf harvested at the onset of flowering. Illinois Protein Strain and cycle (65 or 105) are indicated. Strains included Illinois High Protein (IHP) and Illinois Low Protein (ILP). Illinois High Oil (IHO) is used as a control.

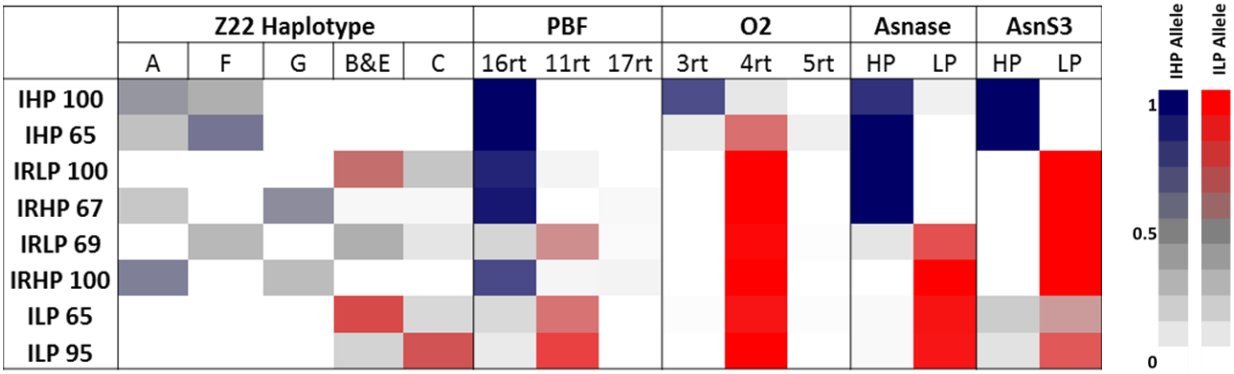


Figure 3.4. Haplotype and allele frequency heat map in 24 individuals from Illinois Protein Strains at cycles 65 and 100. Strains included Illinois High Protein (IHP), Illinois Low Protein (ILP), Illinois Reverse High Protein (IRHP) and Illinois Reverse Low Protein (IRLP). Genes surveyed include *Prolamin-box factor (PBF)*, *Opaque2* (*O2)*, *Asparaginase (ASNase)*, and *Asparagine synthetase3* (*AsnS3)*. Additionally, five haplotypes were discovered from genotyping twelve 22-kD α-zein genes, including A, F, G, B&E, and C. Cycle-strain combinations are indicated on the left and ordered according to protein concentration from highest (IHP100) to lowest (ILP95). IHP alleles indicated in blue, and ILP alleles indicated in red.

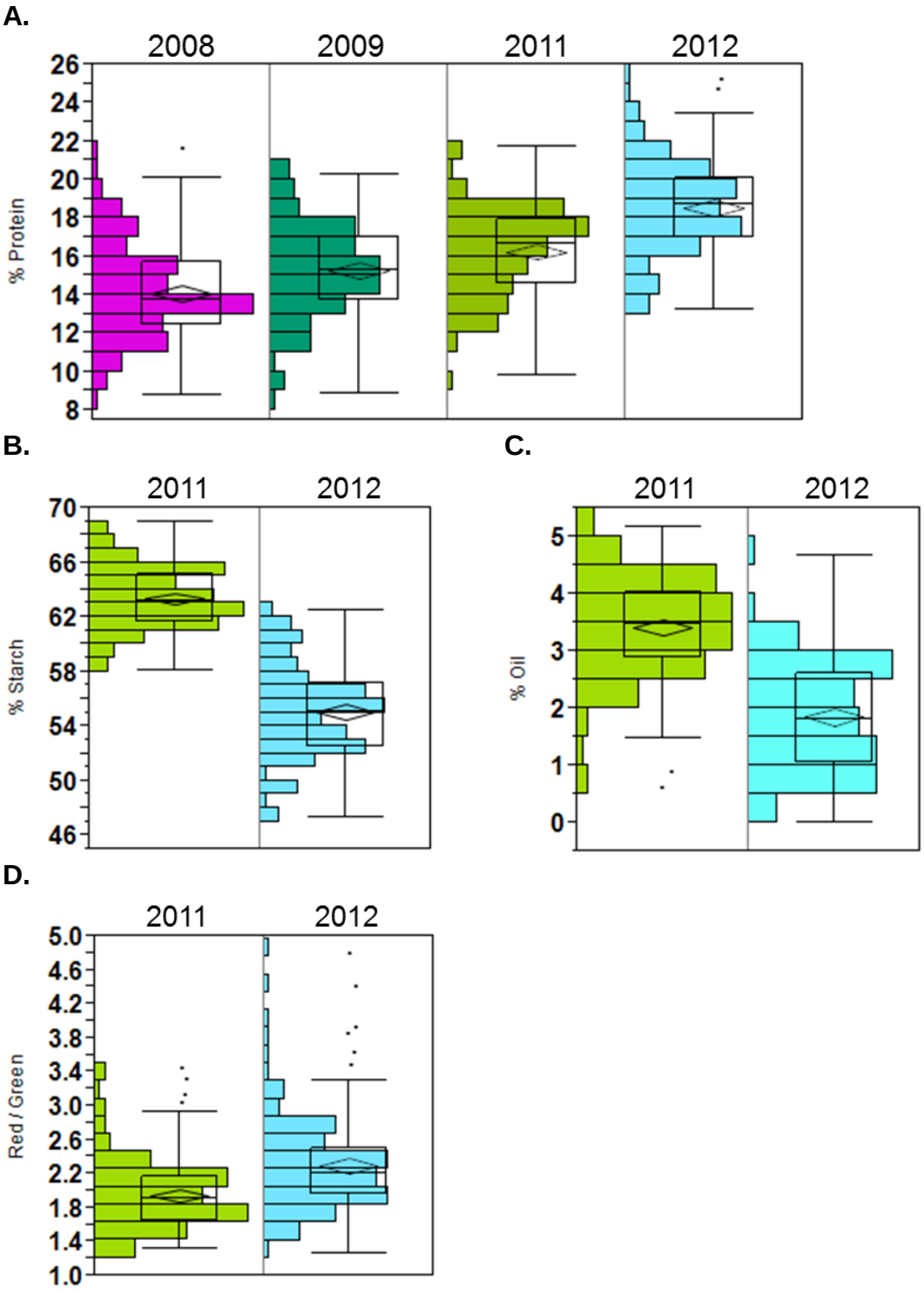


Figure 3.5. Histograms and box and whisker plots of kernel composition traits measured in the IPSRI population grown in 2008, 2009, 2011 and 2012. Traits measured include A. protein %, B. starch %, C. oil % and D. the Axiovision Red/Green (R/G) phenotype.

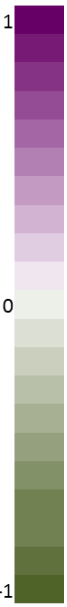
Tables

Table 3.1. ANOVA results showing the effects of genotype, year, ear replication (Earrep), and the interaction of genotype and year on kernel composition traits in the IPSRI population grown in 2008, 2009, 2011 and 2012. Years included in the analysis are indicated in parenthesis. R/G indicates the Axiovision Red/Green (R/G) phenotype. The broad sense heritabilities of the traits were calculated from the ANOVA data.

		Variance Component				Heritability
Trait		Genotype	Year	Earrep	G*Y	
Protein (08,9,11,12)	Fvalue	11.6	364.81	2.77	1.8	0.55
	Pr>F	<0.0001	<0.0001	0.1	<0.0001	
Starch (11,12)	Fvalue	9.94	3133	21.35	2.55	0.53
	Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	
Oil (11,12)	Fvalue	4.13	588	29.79	2.67	0.2
	Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	
R/G (11,12)	Fvalue	9.02	204.12	0.88	1.81	0.63
	Pr>F	<0.0001	<0.0001	0.3489	<0.0001	

Table 3.2. Results of Pearson's Correlation analysis of kernel composition traits in the IPSRI grown in 2008, 2009, 2011 and 2012. Traits included protein, starch and oil concentrations, and the *Floury2*-mRFP1 Red/Green (R/G) phenotype. Correlations are color coded according to magnitude from -1 (green) to 0 (white) to 1 (purple). P-values are reported below the correlations.

	2008_PROTEIN	2009_PROTEIN	2011_PROTEIN	2012_PROTEIN	2011_STARCH	2012_STARCH	2011_OIL	2012_OIL	2011_R/G	2012_R/G
2008_PROTEIN	1.00	0.65	0.64	0.52	-0.59	-0.54	0.01	-0.18	0.28	0.21
p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.8712	0.0463	0.0019	0.0211
2009_PROTEIN		1.00	0.58	0.52	-0.57	-0.56	-0.03	-0.18	0.36	0.13
p-value			<0.0001	<0.0001	<0.0001	<0.0001	0.7816	0.0434	<0.0001	0.1454
2011_PROTEIN			1.00	0.52	-0.91	-0.60	-0.06	-0.20	0.48	0.23
p-value				<0.0001	<0.0001	<0.0001	0.4646	0.0261	<0.0001	0.0080
2012_PROTEIN				1.00	-0.48	-0.83	0.04	-0.38	0.37	0.33
p-value					<0.0001	<0.0001	0.6602	<0.0001	<0.0001	0.0002
2011_STARCH					1.00	0.60	0.02	0.14	-0.47	-0.22
p-value						<0.0001	0.8039	0.1332	<0.0001	0.0114
2012_STARCH						1.00	0.08	0.19	-0.29	-0.14
p-value							0.3855	0.0366	0.0013	0.1226
2011_OIL							1.00	0.14	0.01	0.11
p-value								0.1298	0.9445	0.2036
2012_OIL								1.00	-0.03	-0.02
p-value									0.7766	0.8494
2011_R/G									1.00	0.67
p-value										<0.0001
2012_R/G										1.00



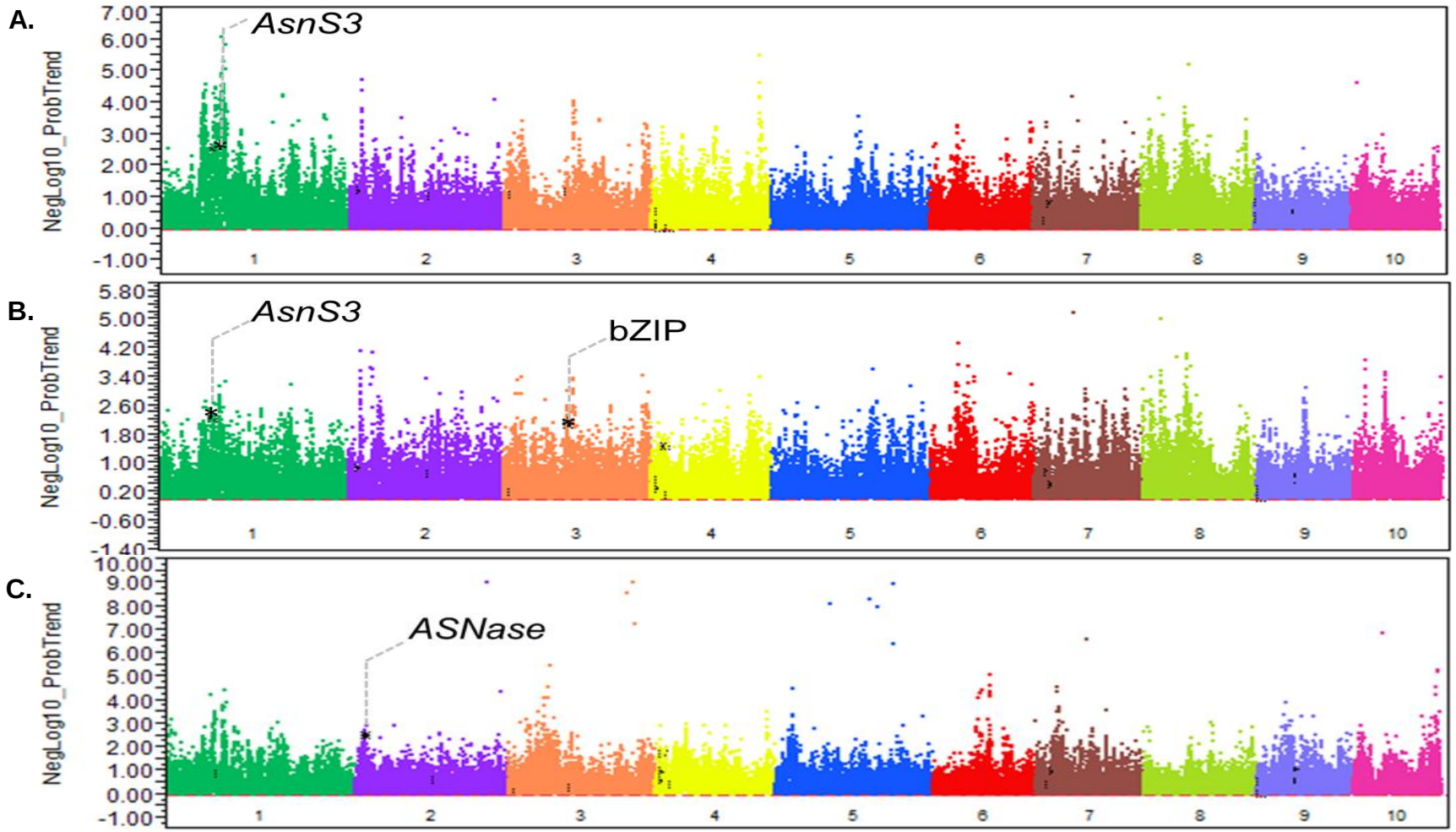


Figure 3.6. Manhattan plots of SNP-Trait Associations between 70K SNP markers and kernel composition phenotypes on the IPSRI mapping population. Traits include **A.** protein concentration **B.** starch concentration and **C.** Axiovision Red/ Green. Protein concentration represents mean phenotypic values for 2008, 2009, 2011, and 2012. Starch concentration and Red/ Green represent mean phenotypic values for 2011 and 2012. Negative log10(pvalues) are indicated on the y-axis. Chromosome number is indicated on the x-axis. The most significant candidate genes are indicated.

Figure 3.7. Manhattan plot of SNP-Trait Associations between 70K SNP markers and duration of juvenile leaf wax measured in the IPSRI mapping population. Negative log10(pvalues) are indicated on the y-axis. Chromosome number is indicated on the x-axis. Molecular markers within the *Glossy15* gene are indicated by asterisks.

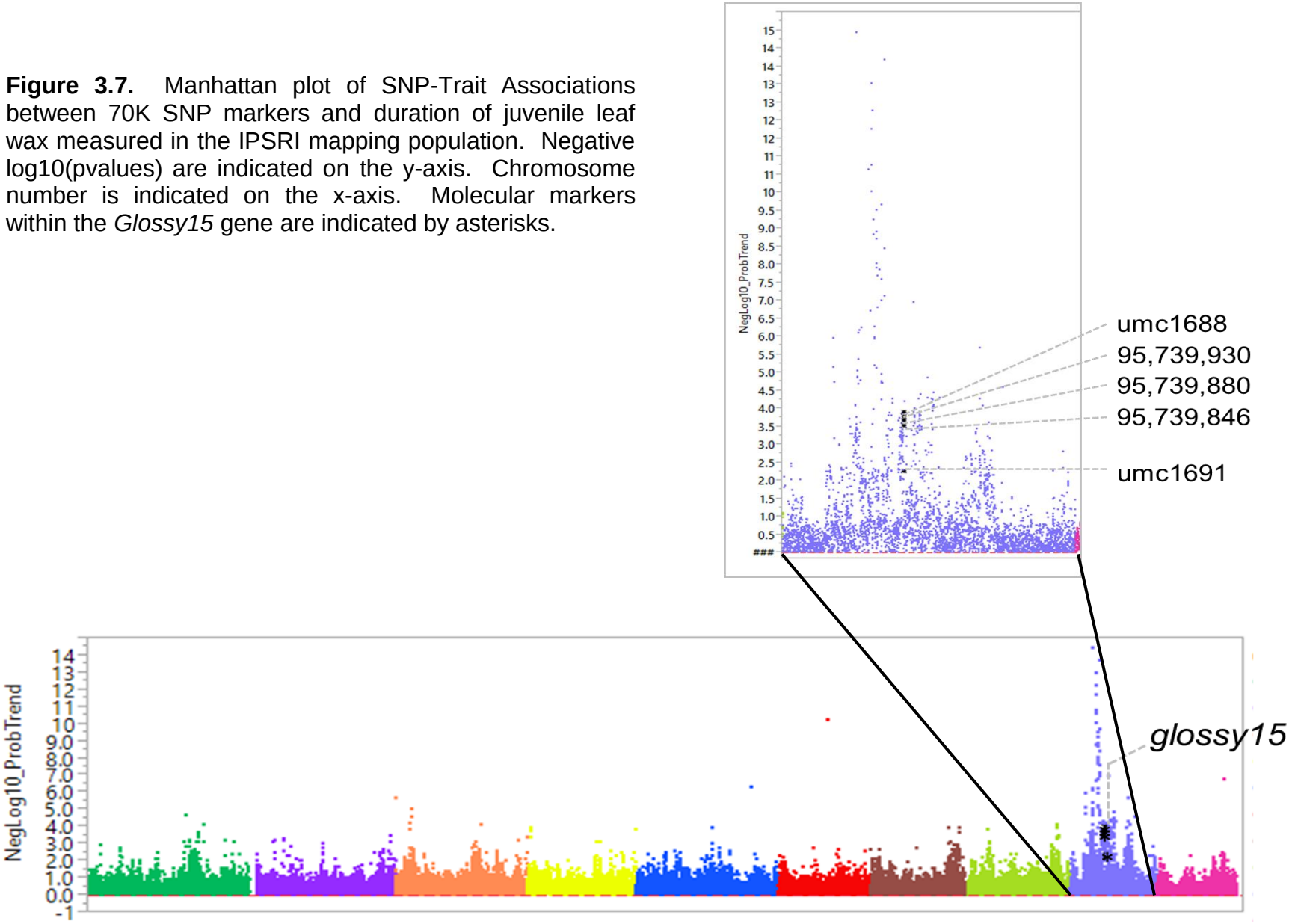


Table 3.3 Results of candidate gene-phenotype associations on the IPSRI mapping population. Traits include protein and starch concentration, the Axiovision Red/ Green phenotype and *glossy15* mutant phenotype. Trait, chromosome, candidate gene marker, marker physical location (BPPos), GRM, gene annotation, F-value, negative log10(pvalue), and R^2 are reported for each candidate gene-phenotype association.

Trait	Chr	Marker	BPPos	GRM	Gene	F-Value	Neg_log10 (pval)	R2
PROT_4YR	1	AsnS3	45114258	GRMZM2G053669	asparagine synthetase3	9.56	2.61	0.072
PROT_4YR	2	ASNase	5068414	GRMZM2G082032	asparaginase	3.66	1.23	0.031
PROT_4YR	2	ASNase3UTR	5073171	GRMZM2G082032	asparaginase	3.19	1.12	0.024
PROT_4YR	2	UMC1065	153510052	GRMZM2G146283	prolamin-box binding factor 1	2.87	1.03	0.021
PROT_4YR	3	bZIP	138878960	GRMZM2G024851	DNA binding protein	3.49	1.19	0.028
PROT_4YR	4	UMC2150	5375803	n/a	within z1A cluster	0.16	0.16	0.001
PROT_4YR	4	UMC1943	5412455	n/a	within z1A cluster	0.02	0.05	0.000
PROT_4YR	4	BNLG0421	20485293	n/a	within z1C cluster	0.01	0.04	0.000
PROT_4YR	7	umc1066	10793341	GRMZM2G015534	opaque2	0.38	0.27	0.003
PROT_4YR	9	umc1688	95739600	GRMZMG160730	glossy15	0.87	0.45	0.007
PROT_4YR	9	umc1691	95742200	GRMZMG160730	glossy15	0.45	0.30	0.004
STA_2YR	1	AsnS3	45114258	GRMZM2G053669	asparagine synthetase3	8.01	2.27	0.061
STA_2YR	2	ASNase	5068414	GRMZM2G082032	asparaginase	2.37	0.90	0.020
STA_2YR	2	ASNase3UTR	5073171	GRMZM2G082032	asparaginase	0.97	0.48	0.007
STA_2YR	2	UMC1065	153510052	GRMZM2G146283	prolamin-box binding factor 1	1.66	0.70	0.013
STA_2YR	3	bZIP	138878960	GRMZM2G024851	DNA binding protein	7.27	2.10	0.057
STA_2YR	4	UMC2150	5375803	n/a	within z1A cluster	0.88	0.45	0.007
STA_2YR	4	UMC1943	5412455	n/a	within z1A cluster	0.40	0.28	0.003
STA_2YR	4	BNLG0421	20485293	n/a	within z1C cluster	0.07	0.10	0.001
STA_2YR	7	umc1066	10793341	GRMZM2G015534	opaque2	1.81	0.74	0.014
STA_2YR	9	umc1688	95739600	GRMZMG160730	glossy15	0.78	0.42	0.006
STA_2YR	9	umc1691	95742200	GRMZMG160730	glossy15	0.36	0.26	0.003
R/G_2YR	1	AsnS3	45114258	GRMZM2G053669	asparagine synthetase3	2.30	0.88	0.018
R/G_2YR	2	ASNase	5068414	GRMZM2G082032	asparaginase	9.18	2.52	0.075
R/G_2YR	2	ASNase3UTR	5073171	GRMZM2G082032	asparaginase	5.92	1.79	0.044
R/G_2YR	2	UMC1065	153510052	GRMZM2G146283	prolamin-box binding factor 1	1.50	0.65	0.011
R/G_2YR	3	bZIP	138878960	GRMZM2G024851	DNA binding protein	0.44	0.29	0.004
R/G_2YR	4	UMC2150	5375803	n/a	within z1A cluster	2.96	1.06	0.022
R/G_2YR	4	UMC1943	5412455	n/a	within z1A cluster	1.51	0.65	0.011
R/G_2YR	4	BNLG0421	20485293	n/a	within z1C cluster	0.89	0.46	0.007
R/G_2YR	7	umc1066	10793341	GRMZM2G015534	opaque2	0.76	0.42	0.006
R/G_2YR	9	umc1688	95739600	GRMZMG160730	glossy15	1.47	0.64	0.011
R/G_2YR	9	umc1691	95742200	GRMZMG160730	glossy15	1.74	0.72	0.016

Table 3.3 continued.

GLOSSY15	1	AsnS3	45114258	GRMZM2G053669	asparagine synthetase3	0.03	0.07	0.000
GLOSSY15	2	ASNase	5068414	GRMZM2G082032	asparaginase	1.07	0.52	0.009
GLOSSY15	2	ASNase3UTR	5073171	GRMZM2G082032	asparaginase	0.03	0.07	0.000
GLOSSY15	2	UMC1065	153510052	GRMZM2G146283	prolamin-box binding factor 1	0.65	0.38	0.005
GLOSSY15	3	bZIP	138878960	GRMZM2G024851	DNA binding protein	1.26	0.58	0.010
GLOSSY15	4	UMC2150	5375803	n/a	within z1A cluster	0.58	0.35	0.004
GLOSSY15	4	UMC1943	5412455	n/a	within z1A cluster	0.38	0.27	0.003
GLOSSY15	4	BNLG0421	20485293	n/a	within z1C cluster	1.96	0.79	0.015
GLOSSY15	7	umc1066	10793341	GRMZM2G015534	opaque2	0.64	0.37	0.005
GLOSSY15	9	umc1688	95739600	GRMZMG160730	glossy15	15.66	3.91	0.107
GLOSSY15	9	umc1691	95742200	GRMZMG160730	glossy15	8.10	2.28	0.069

Table 3.4 Results of SNP-trait associations (FDR α=0.001) using the 70K SNP dataset on the IPSRI mapping population. Traits include protein and starch concentration, the Axiovision Red/ Green phenotype and *glossy15* mutant phenotype. Only SNPs for which a gene was identified within 10Kb up- or down-stream are included. If no GRM was found within 10Kb of a significant SNP, this SNP was included in Supplementary Table 3.1. SNPs that were commonly discovered for more than one trait are highlighted. Trait, chromosome, SNP physical location (BPPos), GRM, gene annotation, basepair position of the gene start and end, major allele, minor allele, F-value, negative log10(pvalue), and R^2 are reported for each SNP-trait association.

Trait	Chr	BPPos	GRM	Annotation	Gene_ Start	Gene_ End	Maj	Min	F-val	NegLog 10(pval)	R2
PROT_4YR	1	33215375	GRMZM2G346861	thaumatin-like protein 1	33214735	33217233	C	T	17.15	4.21	0.12
PROT_4YR	1	33219076	GRMZM2G346861	Same as above	33214735	33217233	A	G	17.61	4.28	0.13
PROT_4YR	1	45919021	GRMZM2G006370	ATP binding protein	45920336	45923687	A	G	18.27	4.44	0.12
PROT_4YR	1	45919042	GRMZM2G006370	Same as above	45920336	45923687	A	G	18.27	4.44	0.12
PROT_4YR	1	45938033	GRMZM2G307992	At:Thioredoxin superfamily protein	45928394	45931891	G	A	16.80	4.14	0.12
PROT_4YR	1	45938033	GRMZM2G308034	DNA binding protein	45938197	45940618	G	A	16.80	4.14	0.12
PROT_4YR	1	45938033	GRMZM2G308034	Same as above	45938700	45940618	G	A	16.80	4.14	0.12
PROT_4YR	1	53422515	GRMZM2G031001	At:Arabidopsis NAC domain containing protein 87	53421632	53423835	A	C	16.39	4.03	0.12
PROT_4YR	1	56569449	GRMZM2G043493	Os:basic helix-loop-helix domain containing protein, expressed	56575210	56577035	G	T	21.05	4.92	0.16
PROT_4YR	1	56569449	GRMZM2G043948	At:Pseudouridine synthase family protein	56569008	56573612	G	T	21.05	4.92	0.16
PROT_4YR	1	56569449	GRMZM2G044038	At:SOS3-interacting protein 1	56564395	56567128	G	T	21.05	4.92	0.16
PROT_4YR	1	56674740	GRMZM2G030571	At:PYRIMIDINE 4	56674097	56676877	C	T	26.73	6.07	0.17
PROT_4YR	1	56893546	GRMZM2G069618	TPR domain containing protein	56892742	56895106	C	T	18.05	4.36	0.14
PROT_4YR	1	56893850	GRMZM2G069618	Same as above	56892742	56895106	G	A	16.73	4.07	0.14
PROT_4YR	1	57249139	GRMZM2G107597	Cytochrome c oxidoreductase	57246486	57249614	T	C	16.58	4.08	0.12
PROT_4YR	1	57249254	GRMZM2G107597	Same as above	57246486	57249614	A	T	16.00	3.97	0.11
PROT_4YR	1	57249255	GRMZM2G107597	Same as above	57246486	57249614	G	C	16.00	3.97	0.11
PROT_4YR	1	57249259	GRMZM2G107597	Same as above	57246486	57249614	G	A	16.00	3.97	0.11
PROT_4YR	1	59959361	GRMZM2G038598	UDP-glucose-4-epimerase	59956283	59958133	G	C	18.86	4.55	0.13
PROT_4YR	1	60095063	GRMZM2G378907	tubby protein	60092678	60100770	G	A	16.17	4.01	0.11
PROT_4YR	1	60264866	GRMZM2G144868	nucleolar GTP-binding protein 2	60261032	60263833	T	A	17.01	4.17	0.12
PROT_4YR	1	60264876	GRMZM2G144868	Same as above	60261032	60263833	A	G	17.01	4.17	0.12
PROT_4YR	1	60864288	GRMZM2G101689	At:WD-40 repeat family protein / small nuclear ribonucleoprotein Prp4p-related	60861638	60865926	A	C	16.67	4.06	0.13

Table 3.4 continued.

PROT_4YR	1	60864288	GRMZM2G101744	Os:R3H domain containing protein, expressed	60867082	60870260	A	C	16.67	4.06	0.13
PROT_4YR	1	61020534	GRMZM2G038153	At:Terpenoid cyclases/Protein prenyltransferases superfamily protein	61012519	61018961	T	C	16.49	4.00	0.15
PROT_4YR	1	61021047	GRMZM2G038153	Same as above	61012519	61018961	A	G	19.03	4.59	0.13
PROT_4YR	1	61448951	GRMZM2G061624	At:Protein with RING/U-box and TRAF-like domains	61451315	61455017	T	G	21.72	5.07	0.16
PROT_4YR	1	61448955	GRMZM2G061624	Same as above	61451315	61455017	G	A	21.72	5.07	0.16
PROT_4YR	1	61448963	GRMZM2G061624	Same as above	61451315	61455017	C	A	21.72	5.07	0.16
PROT_4YR	1	61448976	GRMZM2G061624	Same as above	61451315	61455017	C	T	21.72	5.07	0.16
PROT_4YR	2	5997510	GRMZM2G361388	At:Sterile alpha motif (SAM) domain-containing protein	5998276	5999188	G	C	18.10	4.39	0.13
PROT_4YR	2	5997511	GRMZM2G361388	Same as above	5998276	5999188	G	T	19.66	4.71	0.13
PROT_4YR	2	5997528	GRMZM2G361388	Same as above	5998276	5999188	G	A	18.10	4.39	0.13
PROT_4YR	2	5997536	GRMZM2G361388	Same as above	5998276	5999188	A	G	18.10	4.39	0.13
PROT_4YR	3	150049966	GRMZM2G039867	At:Ankyrin repeat family protein / BTB/POZ domain-containing protein	150049274	150052250	T	C	16.33	4.01	0.13
PROT_4YR	3	151055891	GRMZM2G062524	At:Protein kinase family protein with ARM repeat domain	151047728	151056788	G	A	16.72	4.07	0.14
PROT_4YR	3	151056366	GRMZM2G062524	Same as above	151047728	151056788	C	T	16.05	3.99	0.11
PROT_4YR	4	234447770	GRMZM2G146028	At:ethylene responsive element binding factor 3	234447181	234448465	G	C	16.81	4.14	0.11
PROT_4YR	4	234447772	GRMZM2G146028	Same as above	234447181	234448465	G	T	17.19	4.22	0.12
PROT_4YR	4	234687836	GRMZM2G125308	At:Protein kinase superfamily protein	234687556	234689704	C	T	19.29	4.62	0.14
PROT_4YR	4	234687836	GRMZM2G125345	uncharacterized protein	234681347	234683297	C	T	19.29	4.62	0.14
PROT_4YR	7	118307241	GRMZM2G041163	uncharacterized protein	118307728	118308971	T	C	17.36	4.19	0.14
PROT_4YR	7	118307241	GRMZM2G041175	senescence-associated protein DH	118310662	118312830	T	C	17.36	4.19	0.14
PROT_4YR	7	118307241	GRMZM2G339728	Os:retrotransposon protein, putative, unclassified, expressed	118305189	118306403	T	C	17.36	4.19	0.14
PROT_4YR	8	19834231	GRMZM2G516301	At:WRKY DNA-binding protein 51	19834281	19835285	A	C	17.09	4.16	0.13
PROT_4YR	8	114828477	GRMZM2G084489	At:CW-type Zinc Finger	114826065	114834569	T	G	22.81	5.20	0.19
PROT_4YR	10	6071176	GRMZM5G805685	At:AP2/B3-like transcriptional factor family protein	6070107	6072257	G	C	19.76	4.63	0.17
STA_2YR	1	55503865	GRMZM2G075245	uncharacterized protein	55505245	55505976	G	T	12.06	3.15	0.09
STA_2YR	1	64889748	GRMZM2G306919	PLASTIDIC TYPE I SIGNAL PEPTIDASE 1, PLSP1	64885893	64889912	G	A	12.55	3.26	0.09

Table 3.4 continued.

STA_2YR	2	5997510	GRMZM2G361388	At:Sterile alpha motif (SAM) domain-containing protein	5998276	5999188	G	C	14.08	3.58	0.10
STA_2YR	2	5997511	GRMZM2G361388	Same as above	5998276	5999188	G	T	13.03	3.36	0.09
STA_2YR	2	5997528	GRMZM2G361388	Same as above	5998276	5999188	G	A	14.08	3.58	0.10
STA_2YR	2	5997536	GRMZM2G361388	Same as above	5998276	5999188	A	G	14.08	3.58	0.10
STA_2YR	2	6031061	GRMZM2G022563	At:3-ketoacyl-acyl carrier protein synthase III	6033112	6040532	A	C	16.80	4.13	0.12
STA_2YR	2	6031596	GRMZM2G022563	Same as above	6033112	6040532	C	T	12.19	3.18	0.09
STA_2YR	2	13298791	GRMZM2G038714	uncharacterized protein	13302308	13303290	A	G	14.59	3.66	0.11
STA_2YR	2	13298791	GRMZM2G038722	At:myb domain protein 63	13298750	13300526	A	G	14.59	3.66	0.11
STA_2YR	2	13298800	GRMZM2G038714	uncharacterized protein	13302308	13303290	C	T	12.28	3.18	0.10
STA_2YR	2	13298800	GRMZM2G038722	At:myb domain protein 63	13298750	13300526	C	T	12.28	3.18	0.10
STA_2YR	2	13298804	GRMZM2G038714	uncharacterized protein	13302308	13303290	G	A	14.59	3.66	0.11
STA_2YR	2	13298804	GRMZM2G038722	At:myb domain protein 63	13298750	13300526	G	A	14.59	3.66	0.11
STA_2YR	2	13299879	GRMZM2G038714	uncharacterized protein	13302308	13303290	C	G	13.15	3.39	0.09
STA_2YR	2	13299979	GRMZM2G038722	At:myb domain protein 63	13298750	13300526	A	C	14.50	3.63	0.12
STA_2YR	2	143391114	GRMZM2G135990	At:Protein of unknown function (DUF3049)	143382662	143384017	G	C	13.17	3.35	0.11
STA_2YR	3	12287777	GRMZM2G066362	At:asparagine-linked glycosylation 3	12292951	12297453	T	A	13.33	3.41	0.10
STA_2YR	3	12287777	GRMZM2G066413	At:Glucose-6-phosphate/phosphate translocator-related	12289349	12292072	T	A	13.33	3.41	0.10
STA_2YR	3	12287798	GRMZM2G066362	At:asparagine-linked glycosylation 3	12292951	12297453	A	C	13.33	3.41	0.10
STA_2YR	3	12287798	GRMZM2G066413	At:Glucose-6-phosphate/phosphate translocator-related	12289349	12292072	A	C	13.33	3.41	0.10
STA_2YR	3	150049966	GRMZM2G039867	At:Ankyrin repeat family protein / BTB/POZ domain-containing protein	150049274	150052250	T	C	13.20	3.37	0.11
STA_2YR	3	151056366	GRMZM2G062524	At:Protein kinase family protein with ARM repeat domain	151047728	151056788	C	T	12.71	3.29	0.09
STA_2YR	3	223740841	GRMZM2G157574	etched1	223739197	223740961	T	G	13.70	3.45	0.12
STA_2YR	3	223740841	GRMZM2G157588	At:DNAJ heat shock N-terminal domain-containing protein	223741308	223745402	T	G	13.70	3.45	0.12
STA_2YR	3	223740841	GRMZM2G458095	At:cytochrome BC1 synthesis	223737078	223738942	T	G	13.70	3.45	0.12
STA_2YR	5	183493040	GRMZM2G081955	tab2 protein	183483530	183485782	G	A	14.46	3.60	0.13
STA_2YR	6	82503119	GRMZM2G111906	terminal acidic SANT 1	82497057	82500132	G	C	12.07	3.13	0.10
STA_2YR	6	82503119	GRMZM2G111906	Same as above	82497069	82500119	G	C	12.07	3.13	0.10

Table 3.4 continued.

STA_2YR	6	82503273	GRMZM2G111906	Same as above	82497069	82500119	T	C	18.21	4.34	0.16
STA_2YR	6	99768131	GRMZM2G112337	microtubule-associated protein MAP65-1a	99761240	99767447	T	G	12.36	3.21	0.09
STA_2YR	6	99768209	GRMZM2G112337	Same as above	99767719	99770826	C	A	14.62	3.69	0.10
STA_2YR	6	107881940	GRMZM2G392700	smr domain containing protein	107882001	107883021	C	T	12.76	3.28	0.10
STA_2YR	6	107881989	GRMZM2G392700	Same as above	107882001	107883021	G	A	12.76	3.28	0.10
STA_2YR	6	110437159	GRMZM2G156529	At:WRKY family transcription factor	110436475	110437394	A	G	13.40	3.41	0.11
STA_2YR	6	153240839	AC209257.4_FG006	PF00847: AP2 domain	153233970	153236020	T	A	13.75	3.48	0.11
STA_2YR	7	118307241	GRMZM2G041163	uncharacterized protein	118307728	118308971	T	C	22.62	5.20	0.18
STA_2YR	7	118307241	GRMZM2G041175	senescence-associated protein DH	118310662	118312830	T	C	22.62	5.20	0.18
STA_2YR	7	118307241	GRMZM2G339728	Os:retrotransposon protein, putative, unclassified, expressed	118305189	118306403	T	C	22.62	5.20	0.18
STA_2YR	8	19830105	GRMZM2G516301	At:WRKY DNA-binding protein 51	19834281	19835285	C	G	12.55	3.26	0.09
STA_2YR	8	19834190	GRMZM2G516301	Same as above	19834281	19835285	A	G	13.46	3.44	0.10
STA_2YR	8	19834231	GRMZM2G516301	Same as above	19834281	19835285	A	C	21.48	5.01	0.16
STA_2YR	8	19834394	GRMZM2G516301	Same as above	19834281	19835285	C	G	13.47	3.45	0.10
STA_2YR	8	19961824	GRMZM2G095280	At:Uridine diphosphate glycosyltransferase 74E2	19966938	19969214	A	G	14.02	3.55	0.11
STA_2YR	8	65974200	GRMZM2G021331	ATP synthase beta chain	65969534	65973268	T	A	15.92	3.94	0.12
STA_2YR	8	65974200	GRMZM2G022101	indole-3-acetate beta-glucosyltransferase	65981741	65983349	T	A	15.92	3.94	0.12
STA_2YR	8	101960714	GRMZM2G149808	uncharacterized protein	101958089	101962287	A	G	11.95	3.12	0.09
STA_2YR	8	101960752	GRMZM2G149808	Same as above	101958089	101962287	T	C	11.95	3.12	0.09
STA_2YR	8	101960790	GRMZM2G149808	Same as above	101958089	101962287	A	G	16.19	3.98	0.12
STA_2YR	8	101960795	GRMZM2G149808	Same as above	101958089	101962287	G	A	14.34	3.61	0.11
STA_2YR	8	101961651	GRMZM2G149808	Same as above	101958089	101962287	C	A	13.37	3.41	0.10
STA_2YR	8	114828477	GRMZM2G084489	At:CW-type Zinc Finger	114826065	114834569	T	G	14.97	3.71	0.13
STA_2YR	10	81827916	GRMZM2G325907	myb-like DNA-binding domain containing protein	81827003	81830285	T	C	13.81	3.52	0.10
STA_2YR	10	81827932	GRMZM2G325907	Same as above	81827003	81830285	G	A	13.81	3.52	0.10
STA_2YR	10	81959531	GRMZM2G070780	At:Erythronate-4-phosphate dehydrogenase family protein	81957135	81959818	C	A	13.05	3.33	0.11
STA_2YR	10	148977755	GRMZM2G104658	ATP binding protein	148972118	148974136	T	C	13.49	3.42	0.12
R/G_2YR	1	40424700	GRMZM2G087459	protein kinase APK1A	40421675	40424815	T	G	13.74	3.50	0.10
R/G_2YR	1	40424700	GRMZM2G087600	uncharacterized protein	40415190	40421518	T	G	13.74	3.50	0.10
R/G_2YR	1	49091462	GRMZM2G038015	At:basic region/leucine zipper motif 53	49090944	49091860	G	A	14.94	3.72	0.12
R/G_2YR	1	51008084	GRMZM2G550861	uncharacterized protein	51010321	51011073	G	C	14.43	3.62	0.12

Table 3.4 continued.

R/G_2YR	1	51008088	GRMZM2G550861	Same as above	51010321	51011073	C	T	14.43	3.62	0.12
R/G_2YR	1	51022070	GRMZM2G317160	At:AINTEGUMENTA- ike 5	51016688	51022814	G	T	14.02	3.57	0.10
R/G_2YR	1	52567756	GRMZM2G033570	ETHYLENE-INSENSITIVE3-like 1 protein	52564282	52568326	C	T	14.88	3.74	0.10
R/G_2YR	1	52627046	GRMZM2G174680	lipid binding protein	52625970	52627201	G	C	18.37	4.41	0.14
R/G_2YR	1	52925233	GRMZM2G030272	WRKY55 - superfamily of TFs having WRKY and zinc finger domains	52919938	52921358	G	T	16.12	3.93	0.14
R/G_2YR	1	52925235	GRMZM2G030272	Same as above	52919938	52921358	G	T	16.12	3.93	0.14
R/G_2YR	1	52925236	GRMZM2G030272	Same as above	52919938	52921358	C	T	16.12	3.93	0.14
R/G_2YR	1	52925241	GRMZM2G030272	Same as above	52919938	52921358	A	G	16.12	3.93	0.14
R/G_2YR	2	215520343	GRMZM2G131467	At:RNA-binding (RRM/RBD/RNP motifs) family protein	215513884	215517678	G	T	49.71	9.00	0.42
R/G_2YR	2	215520344	GRMZM2G131467	Same as above	215513884	215517678	G	C	49.71	9.00	0.42
R/G_2YR	2	232364569	GRMZM2G310548	uncharacterized protein	232367455	232368404	T	A	21.89	4.38	0.38
R/G_2YR	2	232364570	GRMZM2G310548	Same as above	232367455	232368404	A	G	21.89	4.38	0.38
R/G_2YR	3	37355993	GRMZM2G132944	uncharacterized protein	37352496	37359548	C	G	16.72	4.11	0.12
R/G_2YR	3	37360035	GRMZM2G132944	Same as above	37352496	37359548	C	T	15.21	3.80	0.11
R/G_2YR	3	37360036	GRMZM2G132944	Same as above	37352496	37359548	G	A	15.21	3.80	0.11
R/G_2YR	3	41737261	GRMZM2G144648	At:Peroxidase superfamily protein	41741731	41743930	T	C	19.05	4.54	0.15
R/G_2YR	3	41737261	GRMZM2G443690	At:phosphoinositide 4-kinase gamma 1	41735719	41737698	T	C	19.05	4.54	0.15
R/G_2YR	3	43360635	GRMZM2G111731	At:myb domain protein 105	43359107	43360826	A	C	16.52	4.08	0.11
R/G_2YR	3	50100592	GRMZM2G119894	At:P-glycoprotein 21	50093119	50099618	G	C	24.10	5.49	0.18
R/G_2YR	3	206605249	GRMZM2G169654	DNA-binding protein RAV1	206600510	206602296	G	A	48.33	8.55	0.44
R/G_2YR	4	184783394	GRMZM2G169564	At:WRKY DNA-binding protein 54	184781651	184783303	A	C	61.72	10.32	0.48
R/G_2YR	4	236885612	GRMZM2G119640	At:Zinc finger C-x8-C-x5-C-x3-H type family protein	236890382	236894009	T	A	14.01	3.54	0.11
R/G_2YR	4	236885612	GRMZM2G419891	polyubiquitin	236879357	236883232	T	A	14.01	3.54	0.11
R/G_2YR	5	9617120	GRMZM2G082343	symbiotic ammonium transporter	9616539	9619038	G	T	13.36	3.38	0.12
R/G_2YR	5	9617135	GRMZM2G082343	Same as above	9616539	9619038	C	G	19.04	4.52	0.15
R/G_2YR	5	9617147	GRMZM2G082343	Same as above	9616539	9619038	C	G	13.41	3.39	0.12
R/G_2YR	5	176989860	GRMZM2G001223	At:myb domain protein 106	176991826	176993860	T	G	43.59	8.27	0.37
R/G_2YR	5	185501519	GRMZM2G027697	R2R3MYB-domain protein Fragment	185497518	185499126	C	A	38.06	7.94	0.26
R/G_2YR	6	112436431	GRMZM2G383680	At:Glutaredoxin family protein	112434261	112435857	T	C	16.88	4.13	0.13
R/G_2YR	6	118087822	GRMZM2G155343	uncharacterized protein	118095262	118096195	G	T	18.64	4.43	0.16
R/G_2YR	7	30505844	GRMZM2G030823	At:F-box/RNI-like/FBD-like domains-containing protein	30504953	30506702	G	A	13.86	3.49	0.12

Table 3.4 continued.

R/G_2YR	7	41419452	GRMZM2G014914	Aquaporin PIP2-1 (Plasma membrane intrinsic protein 2-1)(ZmPIP2-1)(ZmPIP2;1)	41420177	41423983	T	C	14.01	3.51	0.13
R/G_2YR	7	41419530	GRMZM2G014914	Same as above	41420177	41423983	C	T	14.41	3.60	0.13
R/G_2YR	9	40204182	GRMZM2G033029	PPP4R2protein phosphatase 4 core regulatory subunit R2	40203883	40210813	G	A	13.24	3.36	0.12
R/G_2YR	10	80046149	GRMZM2G086496	At:nitrate transporter 1.1	80042433	80048640	G	C	65.61	6.86	0.78
R/G_2YR	10	80046149	GRMZM2G385989	vesicle-associated membrane protein-associated protein A	80051165	80055128	G	C	65.61	6.86	0.78
R/G_2YR	10	147404862	GRMZM2G464137	At:S-methyl-5-thioribose kinase	147402071	147405536	C	A	18.97	4.57	0.13
R/G_2YR	10	147404913	GRMZM2G464137	Same as above	147402071	147405536	G	A	13.02	3.34	0.10
R/G_2YR	10	147404913	GRMZM2G464137	Same as above	147404455	147405547	G	A	13.02	3.34	0.10
R/G_2YR	10	147405087	GRMZM2G464137	Same as above	147402071	147405536	A	G	17.37	4.26	0.12
R/G_2YR	10	147890652	GRMZM2G092571	DHHC zinc finger domain containing protein	147896970	147898723	T	G	22.32	5.21	0.15
R/G_2YR	10	147890784	GRMZM2G092571	Same as above	147896970	147898723	T	C	22.65	5.26	0.16
R/G_2YR	10	148423601	GRMZM2G142743	N-acetylglucosaminyl-phosphatidylinositol de-N-acetylase	148414628	148417925	A	C	13.85	3.52	0.11
GL15	5	204628914	GRMZM2G042027	At:S-adenosylmethionine carrier 1	204624490	204628773	G	A	36.44	6.29	0.49
GL15	5	204628914	GRMZM2G339943	At:plantacyanin	204630114	204631273	G	A	36.44	6.29	0.49
GL15	9	22101585	GRMZM2G064302	Enolase 1 (EC 4.2.1.11)(2-phosphoglycerate dehydratase 1)(2-phospho-D-glycerate hydro-lyase 1)	22100864	22105552	G	A	26.36	5.95	0.18
GL15	9	22105207	GRMZM2G064302	Same as above	22100864	22105552	G	A	22.10	5.15	0.16
GL15	9	22681178	GRMZM5G877500	3-phosphoshikimate 1-carboxyvinyltransferase Fragment (EC 2.5.1.19)	22677939	22681262	G	T	19.73	4.73	0.13
GL15	9	31210325	GRMZM2G308595	nudix hydrolase 4	31205055	31206730	T	C	20.72	4.86	0.16
GL15	9	31210342	GRMZM2G308595	Same as above	31205055	31206730	C	T	20.72	4.86	0.16
GL15	9	31210353	GRMZM2G308595	Same as above	31205055	31206730	C	T	20.72	4.86	0.16
GL15	9	31210354	GRMZM2G308595	Same as above	31205055	31206730	A	C	20.72	4.86	0.16
GL15	9	31210359	GRMZM2G308595	Same as above	31205055	31206730	A	C	20.72	4.86	0.16
GL15	9	31210397	GRMZM2G308595	Same as above	31205055	31206730	G	C	21.97	5.05	0.18
GL15	9	31210404	GRMZM2G308595	Same as above	31205055	31206730	A	G	21.13	4.89	0.18
GL15	9	45578052	GRMZM2G050641	At:diacylglycerol acyltransferase family	45577047	45580698	G	T	56.47	10.63	0.36
GL15	9	55347533	GRMZM2G148060	At:iron regulated 2	55346546	55354836	G	A	61.65	11.75	0.34

Table 3.4 continued.

GL15	9	55347756	GRMZM2G148060	Same as above	55346546	55354836	A	T	64.16	12.28	0.33
GL15	9	56423977	GRMZM2G126900	At:myo-inositol oxygenase 1	56422201	56422862	G	T	44.72	8.82	0.32
GL15	9	56423978	GRMZM2G126900	Same as above	56422201	56422862	G	C	44.72	8.82	0.32
GL15	9	56423979	GRMZM2G126900	Same as above	56422201	56422862	G	A	44.72	8.82	0.32
GL15	9	56423983	GRMZM2G126900	Same as above	56422201	56422862	T	C	44.72	8.82	0.32
GL15	9	56423986	GRMZM2G126900	Same as above	56422201	56422862	T	G	44.72	8.82	0.32
GL15	9	56423988	GRMZM2G126900	Same as above	56422201	56422862	G	C	44.72	8.82	0.32
GL15	9	56424613	GRMZM2G126900	Same as above	56422201	56422862	C	A	44.86	9.26	0.25
GL15	9	57012019	GRMZM2G113203	PF00664: ABC transporter transmembrane region	57011529	57017702	C	A	26.43	5.97	0.18
GL15	9	57012030	GRMZM2G113203	Same as above	57011529	57017702	G	A	26.00	5.93	0.17
GL15	9	57012525	GRMZM2G113203	Same as above	57011529	57017702	T	C	22.27	5.21	0.15
GL15	9	57012530	GRMZM2G113203	Same as above	57011529	57017702	T	C	22.27	5.21	0.15
GL15	9	57012873	GRMZM2G113203	Same as above	57011529	57017702	G	A	26.15	5.94	0.17
GL15	9	57012908	GRMZM2G113203	Same as above	57011529	57017702	C	T	27.74	6.26	0.17
GL15	9	59400919	GRMZM2G476230	At:Protein of unknown function (DUF761)	59400775	59402188	G	C	37.28	7.89	0.24
GL15	9	59400939	GRMZM2G476230	Same as above	59400775	59402188	G	A	42.91	8.90	0.25
GL15	9	59830635	GRMZM2G080689	peroxidase 52	59829875	59831505	A	T	35.68	7.69	0.21
GL15	9	59848097	GRMZM2G380247	At:Peroxidase superfamily protein	59840503	59842682	T	G	31.34	6.81	0.22
GL15	9	60761873	GRMZM2G395458	At:CBL-interacting protein kinase 18	60761293	60762717	T	C	93.13	15.16	0.49
GL15	9	60761924	GRMZM2G395458	Same as above	60761293	60762717	T	C	37.92	7.86	0.27
GL15	9	64295850	GRMZM2G148400	At:Glycosyl hydrolase superfamily protein	64295120	64299369	T	C	20.94	4.96	0.14
GL15	9	64295871	GRMZM2G148400	Same as above	64295120	64299369	G	A	19.99	4.75	0.14
GL15	9	73475848	GRMZM2G350918	At:Protein kinase family protein with leucine-rich repeat domain	73472167	73476083	G	A	75.62	13.69	0.38
GL15	9	73475848	GRMZM2G350931	uncharacterized protein	73472558	73473268	G	A	75.62	13.69	0.38
GL15	9	100992537	GRMZM2G360523	At:response regulator 12	100983990	100992750	A	T	32.24	6.96	0.22
GL15	10	143500872	GRMZM2G148621	cyclin-dependent protein kinase	143500958	143501803	C	A	31.16	6.73	0.23
GL15	10	143500872	GRMZM2G148633	At:peroxin 22	143504901	143505681	C	A	31.16	6.73	0.23

Table 3.5 Summary results of IPSRI linkage map with high confidence marker data set. Average basepairs per cM, total centiMorgans (cM) and number of genetic markers are reported on a per chromosome basis.

Chromosome	Average basepairs per cM	cM	Number markers
1	373585	1132.52	637
2	572087	618.93	317
3	429526	627.58	482
4	429852	682.51	513
5	352767	864.89	353
6	436828	505.89	298
7	443716	444.47	332
8	403429	454.28	335
9	414294	545.73	148
10	537608	390.56	264
Total		**6267.36**	**3679**

Figure 3.8 IPSRI linkage map with high confidence marker data set.

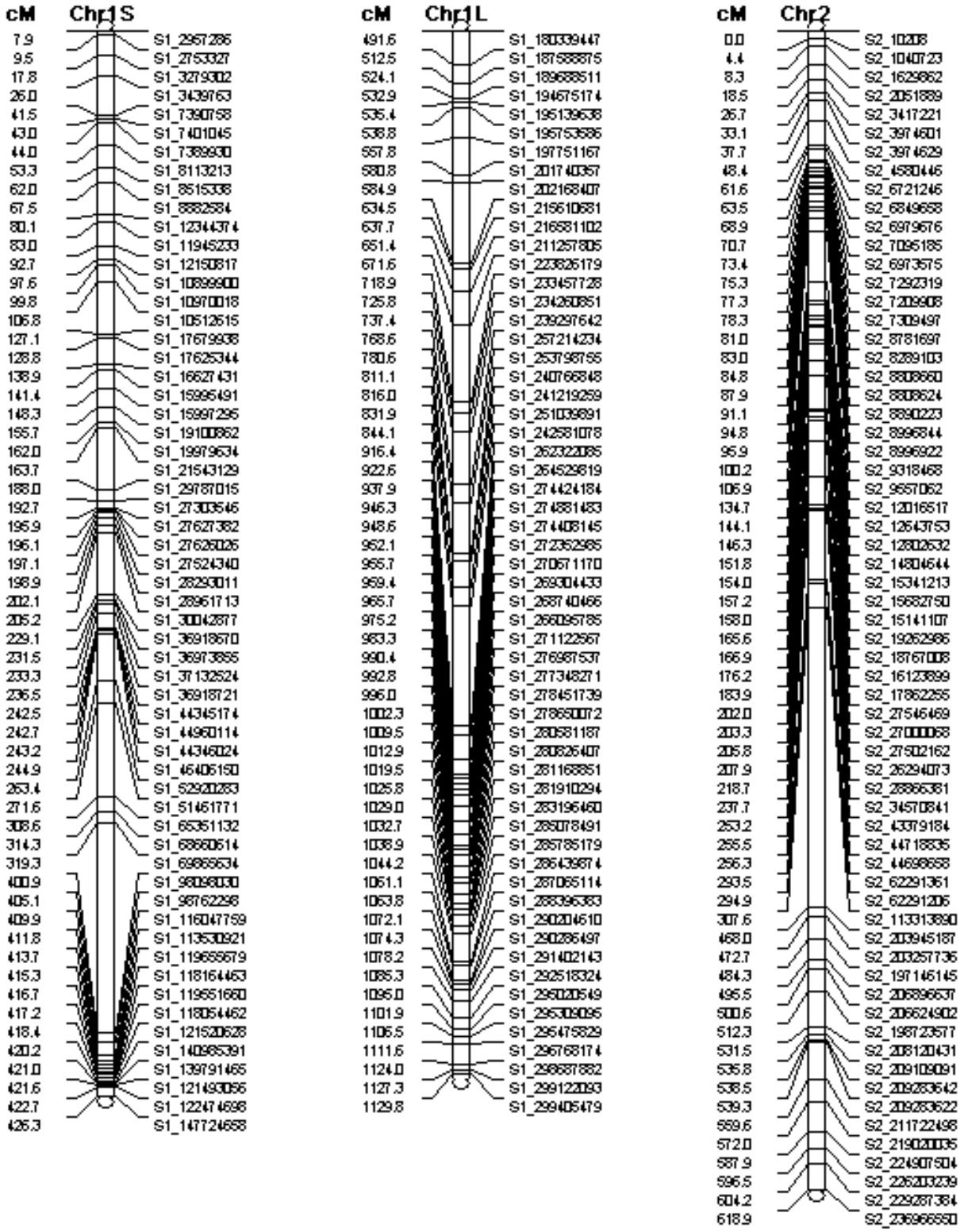


Figure 3.8 continued.

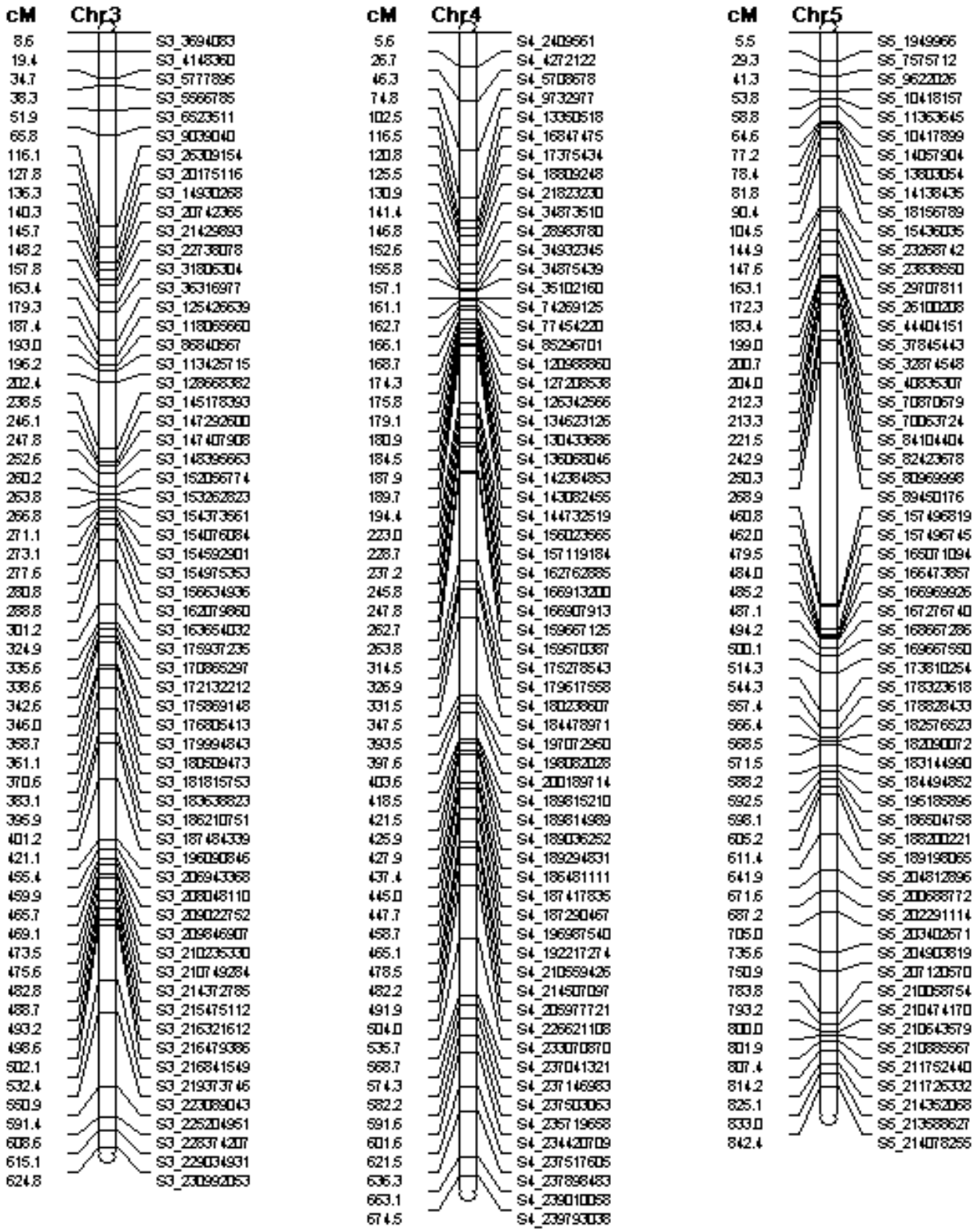


Figure 3.8 continued.

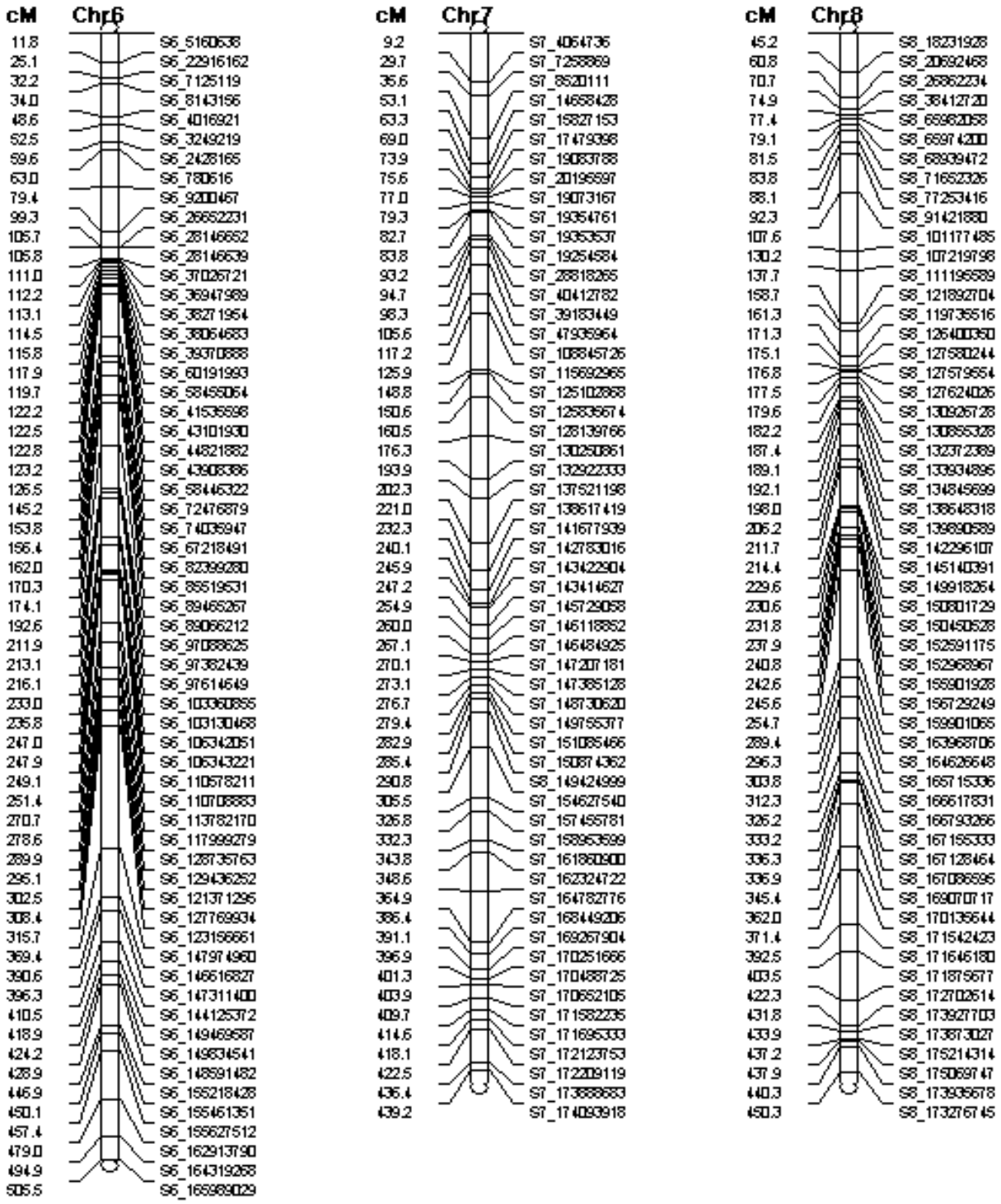
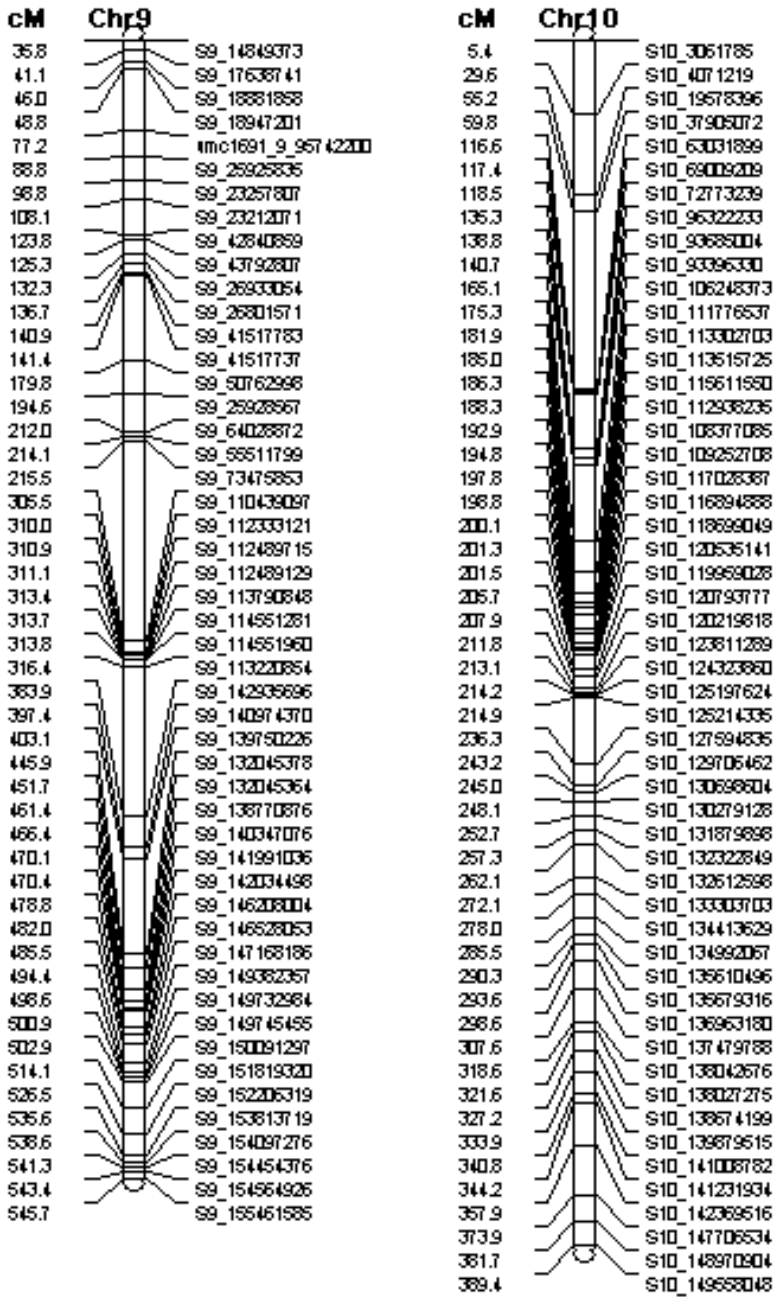


Figure 3.8 continued.



References

Barniex, A.J. Physiology and biochemistry of source-regulated protein accumulation in the wheat grain (2007) *Journal of Plant Physiology*, 164: 581-590.

Below, F.E., J.R. Seebauer, M. Uribelarrea, M.C. Schneerman, and S.P. Moose (2004) Physiological changes accompanying long-term selection for grain protein in maize. *Plant Breeding Reviews.* 24 (1): 133-151.

Church, J.B. (2008) Functional genomics of nitrogen use in maize. Ph.D. dissertation, University of Illinois.

Clark, R. M., T. N. Wagler, P. Quijada & J. Doebley (2006) A distant upstream enhancer at the maize domestication gene tb1 has pleiotropic effects on plant and inflorescent architecture. *Nature Genetics,* 38**,** 594-597.

Dembinski, E. and S. Bany (1991) The amino acid pool of high and low protein rye inbred lines (*Secale cereal L.)*. *Plant Physiology.* 138: 494-496.

Dembinski, E., Rafalski, A., Wisniewska, I. (1991) Effect of long-term selection for high and low protein content on the metabolism of amino acids and carbohydrates in maize kernel. *Plant Physiology and Biochemistry.* 29 (6): 549-557.

Dembinski E, S. Bany and Raczynska-Bojanowska (1995) Asparagine and glutamine in the leaves of high and low protein maize. ACTA Physiologiae Plantarum. 17(4):361-365.

Dijkhuizen, A., J. W. Dudley, T. R. Rocheford, A. E. Haken & S. R. Eckhoff (1998) Near-infrared reflectance correlated to 100-g wet-milling analysis in maize. *Cereal Chemistry,* 75**,** 266-270.

Dudley, J. W. 1977. Seventy-six generations of selection for oil and protein percentage in maize. In *Proc. Intl. Conf. on Quantitative Genetics,* ed. O. K. E. Pollak, and T.B. Bailey, Jr. , 459-473. Iowa State University Press.

Dudley, J. W. (1994) LINKAGE DISEQUILIBRIUM IN CROSSES BETWEEN ILLINOIS MAIZE STRAINS DIVERGENTLY SELECTED FOR PROTEIN PERCENTAGE. *Theoretical and Applied Genetics,* 87**,** 1016-1020.

Dudley, J. W., D. Clark, T. R. Rocheford & J. R. LeDeaux (2007) Genetic analysis of corn kernel chemical composition in the random mated 7 generation of the cross of generations 70 of IHP x ILP. *Crop Science,* 47**,** 45-57.

Dudley, J. W., A. Dijkhuizen, C. Paul, S. T. Coates & T. R. Rocheford (2004) Effects of random mating on marker-QTL associations in the cross of the Illinois High Protein X Illinois Low Protein maize strains. *Crop Science,* 44**,** 1419-1428.

Dudley, J. W. & R. J. Lambert (1992) 90-Generations of Selection for Oil and Protein in Maize. *Maydica,* 37**,** 81-87.

Elshire, R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler & S. E. Mitchell (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One,* 6**,** e19379.

Fong, S. S., A. R. Joyce & B. O. Palsson (2005) Parallel adaptive evolution cultures of Escherichia coli lead to convergent growth phenotypes with different gene expression states. *Genome Research,* 15**,** 1365-1372.

Forde, B. G., A. Heyworth, J. Pywell & M. Kreis (1985) Nucleotide sequence of a B1 hordein gene and the identification of possible upstream regulatory elements in endosperm storage protein genes from barley, wheat and maize. *Nucleic Acids Research,* 13**,** 7327-39.

Fu, Y., T. Wen, Y.I. Ronin, H.D. Chen, L. Guo, D.I. Mester, Y. Yang, M. Lee, A.B. Korol, D.A. Ashlock, and P.S. Schnable (2006) IBM Genetic Dissection of Intermated Recombinant Inbred Lines Using a New Genetic Map of Maize. *Genetics*, 164: 1671-1683.

Glaubitz, J.C., T.M. Casstevens, F. Lu, J. Harriman, R.J.Elshire, Q. Sun, E.S. Buckler (2014) TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS One,* 9(2): e90346.

Goldman, I. L., T. R. Rocheford & J. W. Dudley (1993) QUANTITATIVE TRAIT LOCI INFLUENCING PROTEIN AND STARCH CONCENTRATION IN THE ILLINOIS LONG-TERM SELECTION MAIZE STRAINS. *Theoretical and Applied Genetics,* 87**,** 217-224.

Gutierez, R. A., T. L. Stokes, K. Thum, X. Xu, M. Obertello, M. S. Katari, M. Tanurdzic, A. Dean, D. C. Nero, C. R. McClung & G. M. Coruzzi (2008) Systems approach identifies an organic nitrogen-responsive gene network that is regulated by the master clock control gene CCA1. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 4939-4944.

Hartings, H., N. Lazzaroni, V. Rossi, G. R. Riboldi, R. D. Thompson, F. Salamini & M. Motto (1995) Molecular Analysis of Opaque-2 Alleles from Zea-Mays L Reveals the Nature of Mutational Events and the Presence of a Hypervariable Region in the 5' Part of the Gene. *Genetical Research,* 65, 11-19.

Henry, A. M. & C. Damerval (1997) High rates of polymorphism and recombination at the Opaque-2 locus in cultivated maize. *Molecular & General Genetics,* 256**,** 147-157.

Hernandez-Sebastia, C., Marsolais, F., Saravitz, C., Israel, D., Dewey, R.E. and Huber, S.C. (2005) Free amino acid profiles suggest a possible role for asparagine in the control of storage-product accumulation in developing seeds of low- and high-protein soybean lines *J. Exp. Bot.* 56: 1951-1963.

Hirsch, C.H., J.M. Foerster, J.N. Johnson, R.S. Sekhorn, G. Muttoni, B. Vaillancourt, F. Peñagaricarno, E. Lindquist, M.A. Pedraza, K. Barry, M. de Leon, S.M. Kaeppler, and C.R. Buell (2014) Insights into the maize pan-genome and pan-transcriptome. *The Plant Cell,* 26: 121-135.

Hoener and DeTurk (1938) The absorption and utilization of nitrate nitrogen during vegetative growth by IHP and ILP corn. *J. American Society of Agronomy.* 30(3).

Kirsten, W.J and G.U Hesselius (1983) Rapid, automatic, high capacity dumas determinatino of nitrogen. *Microchemical Journal,* 28: 529-547.

Lawrence, CJ, Q. Dong, M.L. Polacco, T.E. Seigfried, and V. Brendel. (2004) MaizeGDB, the community database for maize genetics and genomics. *Nucleic Acids Research* 32:D393-D397.

Lee, M.N., N. Sharopova, W.D. Beavis, D. Grant, M. Katt, D. Blair, and A. Hallauer (2002) Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Molecular Biology*, 8: 453-461.

Letchworth, M.B. and R.J. Lambert (1998) Pollen parent effects on oil, protein, and starch concentration in maize kernels. *Crop Science*. 38(2):363-367.

Lohaus, G., M. Buker, M. Hussmann, C. Soave & H. W. Heldt (1998) Transport of amino acids with special emphasis on the synthesis and transport of asparagine in the Illinois low protein and Illinois high protein strains of maize. *Planta,* 205, 181-188.

Lorenzoni C, Alberi, P., Viotti, A., Soave, C., Di Fonzo, N., Gentinetta, E., Maggiore, T and Salamini, F. (1978) Biochemical chracterization of high and low protein strains of maize. *In* Miflin and Zoschke ,eds, Carbohydrate and protein synthesis. Commission of the European Communities, Brussels, pp 173-197.

Lucas, C.J., H. Zhao, M. Schneerman S.P. Moose (2013) Changes in response to 110 cycles of selection for seed protein and oil concentration in maize. *In* Philip W. Becraft, ed, *Seed Genomics*. Wiley-Blackwell.

Marzabal, P., E. Gas, P. Fontanet, J. Vicente-Carbajosa, M. Torrent & M. D. Ludevid (2008) The maize Dof protein PBF activates transcription of gamma-zein during maize seed development. *Plant Molecular Biology,* 67**,** 441-454.

Mohanty, A., Luo, A., DeBlasio, S., Ling, X., Yang, Y. Tuthill, D.E., Williams, K.E., Hill, D., Zadrozny, T., Chan, A., Sylvester, A.W., and Jackson, D. (2009) Advancing cell biology and functional genomics in maize using fluorescent protein-tagged lines. *Plant Physiology*. 149: 601-605.

Moose S.P., Dudley J.W., Rocheford, T.R. (2004) Maize selection passes the century mark: a unique resource for 21st century genomics. *Trends in Plant Science,* 9: 358-364.

Moose, S. P. & R. H. Mumm (2008) Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiology,* 147**,** 969-77.

Moose, S.P. and P.H. Sisco (1994) Glossy15 controls the epidermal juvenile-to-adult phase transition in maize. *Plant Cell,* 6:1343-1355.

Munos, S., C. Cazettes, C. Fizames, F. Gaymard, P. Tillard, M. Lepetit, L. Lejay and A. Gojon (2004) Transcript profiling in the *chl1-5* mutant of Arabidopsis reveals a role of the nitrate transporter NRT1.1 in the regulation of another nitrate transporter, NRT2.1. *Plant Cell,* 16: 2433:2447.

Remans, T., P. Nacry, M. Pervent, S. Filleur, E. Diatloff, E. Mounier, P. Tillard, B. Forde and A. Gojon (2006) The arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. *PNAS,* 103(50): 19206-19211.

Reggiani, R., and Soave, C. (1985) Factors affecting starch and protein content in developing endosperms of high and low protein strains of maize. *Genetica Agrarica.* 39: 221-232.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *PNAS* 81:8014-8018.

Schmidt, R. J., M. Ketudat, M. J. Aukerman & G. Hoschek (1992) Opaque-2 Is a Transcriptional Activator That Recognizes a Specific Target Site in 22-Kd-Zein Genes. *Plant Cell,* 4**,** 689-700.

Schnable, P. S. et al. (2009) The B73 maize genome: complexity, diversity, and dynamics. *Science,* 326: 1112-1114.

Seebauer, J.R., S.P. Moose, B.J. Fabbri, L.D. Crossland and F.E. Below (2004) Amino acid metabolism in maize earshoots, implications for assimilate preconditioning and nitrogen signaling. *Plant Physiology,* 136: 4326-4334.

Sene M., C. Thevenot, D. Hoffman, F. Benetrix, M. Causse, J.-L. Prioul. (2000) QTLs for grain dry milling properties, composition and vitreousness in maize recombinant inbred lines. *Theor. Appl. Genet.* 102: 591-599.

Swanson-Wagner, R. A., Y. Jia, R. DeCook, L. A. Borsuk, D. Nettleton & P. S. Schnable (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F-1 hybrid and its inbred parents. *Proceedings of the National Academy of Sciences of the United States of America,* 103, 6805-6810.

Todd, J., S. Screen, J. Crowley, J. Peng, S. Anderson, T. Brown, Q. Qi, B. Fabbri, S. Duff. (2008) Identification and characterization of four distinct asparagine synthetase (*AsnS*) genes in maize (*Zea mays L.*). *Plant Science*, 175: 799-808.

Topp, C., P.N. Benfey, S. P. Moose and J. W. Edwards (2013). Live 3D imaging reveals the effects of long-term recurrent selection for yield traits on maize root system architecture. 55th Annual Maize Genetics Conference. St. Charles, IL.

Tsai, C.L., Dweikat, I. and Tsai, C.Y. (1990) Effects of source supply and sink demand on the carbon and nitrogen ratio in maize kernels. *Maydica* 35: 391-397.

Uribelarrea M, Below FE, Moose SP (2004) Grain composition and productivity of maize hybrids derived from the Illinois protein strains in response to variable nitrogen supply. *Crop Science*. 44: 1593-1600.

Uribelarrea, M., Moose, S.P., and Below, F.E. (2007) Divergent selection for grain protein affects nitrogen use efficiency in maize hybrids. *Field Crops Res*. 100: 82-90.

Van Ooijen, J.W. (2006) JoinMap 4, software for theh calculatin of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands.

Vicente-Carbajosa, J., S. P. Moose, R. L. Parsons & R. J. Schmidt (1997) A maize zinc-finger protein binds the prolamin box in zein gene promoters and interacts with the basic leucine zipper transcriptional activator Opaque2. *Proceedings of the National Academy of Sciences of the United States of America,* 94**,** 7685-7690.

Wallace, J. C., M. A. Lopes, E. Paiva & B. A. Larkins (1990) New Methods for Extraction and Quantitation of Zeins Reveal a High Content of Gamma-Zein in Modified Opaque-2 Maize. *Plant Physiology,* 92**,** 191-196.

Willmot, D.B., J.W. Dudley, T. R. Rocheford, and A. Bari (2006) Effect of random mating on Marker-QTL Associations for grain quality traits in the cross of Illinois High Oil X Illinois Low Oil. *Maydica* 51:187-199.

Wilson, L.M., S.R. Whitt, A.M. Ibanez, T.R. Rocheford, M.M. Goodman, and E.S. Buckler IV. (2004). Dissection of maize kernel composition and starch production by candidate gene association. *Plant Cell,* 16: 2719-2733.

Wrage, E. (2005) Characterization of the alpha-zein storage proteins of the Illinois maize long-term selection strains. M.S. thesis. University of Illinois at Urbana-Champaign.

Appendix A- Supplementary Tables

Table A.1. Results of SNP-trait associations (FDR α=0.001) using the 70K SNP dataset on the IPSRI mapping population. Traits include protein and starch concentration, the Axiovision Red/ Green phenotype and *glossy15* juvenile leaf wax. Only SNPs for which no gene was identified within 10Kb up- or down-stream are included. Trait, chromosome, SNP physical location (BPPos), major allele, minor allele, F-value, negative log10(pvalue), and R^2 are reported for each SNP-trait association.

Trait	Chr	BPPos	Maj	Min	F-Value	Neg_log 10(pval)	R2
PROT_4YR	1	33162243	C	T	17.57	4.30	0.12
PROT_4YR	1	33162287	C	T	17.10	4.20	0.12
PROT_4YR	1	34210868	T	A	16.77	4.13	0.11
PROT_4YR	1	34960438	G	C	18.24	4.41	0.13
PROT_4YR	1	34960439	T	G	18.24	4.41	0.13
PROT_4YR	1	34961408	C	G	19.04	4.56	0.14
PROT_4YR	1	45403250	C	A	17.75	4.30	0.13
PROT_4YR	1	45905742	T	C	18.52	4.48	0.13
PROT_4YR	1	52605355	C	G	16.68	4.09	0.12
PROT_4YR	1	53413513	C	G	16.63	4.06	0.13
PROT_4YR	1	56929936	C	T	17.28	4.24	0.12
PROT_4YR	1	56970070	A	T	16.89	4.15	0.12
PROT_4YR	1	56970072	A	G	17.11	4.20	0.12
PROT_4YR	1	56970125	C	T	18.92	4.51	0.15
PROT_4YR	1	58459458	A	G	20.37	4.84	0.14
PROT_4YR	1	58473259	G	C	20.43	4.86	0.14
PROT_4YR	1	58739851	T	C	18.53	4.49	0.12
PROT_4YR	1	59122249	G	T	16.53	4.05	0.13
PROT_4YR	1	59462758	G	A	16.00	3.98	0.11
PROT_4YR	1	59462759	T	C	16.00	3.98	0.11
PROT_4YR	1	59577117	G	A	18.11	4.40	0.12
PROT_4YR	1	60755570	A	G	17.71	4.31	0.12
PROT_4YR	1	60808690	A	C	22.69	5.29	0.15
PROT_4YR	1	61134781	A	G	16.26	4.00	0.12
PROT_4YR	1	61134807	T	A	16.26	4.00	0.12
PROT_4YR	1	61624185	C	T	26.28	5.86	0.20
PROT_4YR	1	210137537	A	G	17.38	4.24	0.12
PROT_4YR	1	210137579	C	G	17.20	4.21	0.12
PROT_4YR	2	226677150	T	A	16.95	4.11	0.14
PROT_4YR	3	150769479	G	A	16.42	4.05	0.12
PROT_4YR	4	234621748	G	A	23.90	5.52	0.16
STA_2YR	1	228006479	T	C	12.27	3.20	0.09
STA_2YR	2	5721523	T	C	12.75	3.15	0.18
STA_2YR	2	14207050	G	C	16.63	4.06	0.13

Table A.1 continued.

STA_2YR	3	9935624	T	C	13.02	3.33	0.11
STA_2YR	4	234621748	G	A	13.33	3.42	0.10
STA_2YR	5	209000196	A	G	12.12	3.15	0.10
STA_2YR	6	79983499	G	A	12.12	3.16	0.09
STA_2YR	6	81546687	G	C	12.54	3.24	0.10
STA_2YR	6	82378485	C	A	14.84	3.74	0.10
STA_2YR	6	82399280	G	A	14.86	3.73	0.11
STA_2YR	6	167031862	A	G	12.16	3.17	0.09
STA_2YR	8	10989813	G	C	13.36	3.40	0.11
STA_2YR	8	19295622	G	T	12.39	3.23	0.09
STA_2YR	8	101757452	T	A	12.19	3.18	0.09
STA_2YR	8	102107707	C	T	16.48	4.00	0.14
STA_2YR	8	102107725	C	A	16.48	4.00	0.14
STA_2YR	8	102138297	G	T	15.84	3.93	0.11
STA_2YR	8	102138327	G	C	16.41	4.04	0.12
STA_2YR	10	10394244	G	C	12.01	3.13	0.09
STA_2YR	10	10446844	A	G	14.36	3.60	0.12
STA_2YR	10	10446850	C	A	12.08	3.13	0.10
STA_2YR	10	10446854	A	G	12.08	3.13	0.10
STA_2YR	10	10446886	G	T	12.08	3.13	0.10
STA_2YR	10	10585446	T	A	15.79	3.88	0.13
STA_2YR	10	82028112	G	C	13.59	3.44	0.12
R/G_2YR	1	34947864	G	A	19.90	4.24	0.32
R/G_2YR	1	40436870	C	A	13.72	3.46	0.12
R/G_2YR	1	40436872	C	G	13.95	3.51	0.12
R/G_2YR	1	40436956	C	T	13.58	3.46	0.10
R/G_2YR	1	40437387	A	C	13.05	3.35	0.10
R/G_2YR	1	48999886	G	A	15.46	3.81	0.13
R/G_2YR	3	26192868	G	A	13.68	3.49	0.10
R/G_2YR	3	28140269	T	C	13.95	3.52	0.12
R/G_2YR	3	212406346	C	T	46.16	9.02	0.33
R/G_2YR	3	213412619	T	C	36.34	7.22	0.33
R/G_2YR	5	8426641	G	T	13.34	3.42	0.10
R/G_2YR	5	69458135	G	A	48.24	8.09	0.50
R/G_2YR	5	199721322	T	C	49.33	8.95	0.41
R/G_2YR	5	199721323	G	T	38.99	6.38	0.53
R/G_2YR	6	112602388	C	T	17.68	4.30	0.12
R/G_2YR	6	129970076	G	C	17.37	4.25	0.12
R/G_2YR	6	130084931	A	T	18.19	4.35	0.15
R/G_2YR	6	130131005	T	C	17.40	4.20	0.14
R/G_2YR	6	130131301	T	C	19.08	4.60	0.13

Table A.1 continued.

R/G_2YR	6	130132020	A	G	22.15	5.08	0.19
R/G_2YR	7	30664165	T	C	18.90	4.53	0.14
R/G_2YR	7	30677783	A	T	17.93	4.36	0.12
R/G_2YR	7	30677786	C	G	17.93	4.36	0.12
R/G_2YR	7	30677794	G	A	17.93	4.36	0.12
R/G_2YR	7	40999388	T	G	14.82	3.69	0.12
R/G_2YR	7	130887385	T	C	31.76	6.62	0.28
R/G_2YR	7	153855897	T	G	14.35	3.61	0.11
R/G_2YR	9	45848255	G	C	15.54	3.88	0.11
GLOSSY15	1	196051518	T	C	20.55	4.71	0.20
GLOSSY15	3	1711852	C	A	26.17	5.71	0.24
GLOSSY15	3	12526502	G	A	21.64	5.04	0.16
GLOSSY15	6	128357650	C	A	53.69	10.26	0.34
GLOSSY15	9	30560206	G	A	87.48	14.44	0.48
GLOSSY15	9	31283116	G	C	26.92	6.10	0.17
GLOSSY15	9	31290993	A	T	23.26	5.37	0.17
GLOSSY15	9	33670979	C	T	27.28	6.18	0.17
GLOSSY15	9	33855818	G	A	20.48	4.79	0.16
GLOSSY15	9	34707385	G	A	27.80	6.24	0.18
GLOSSY15	9	49309194	C	G	31.14	6.71	0.23
GLOSSY15	9	55272046	C	T	69.41	13.03	0.35
GLOSSY15	9	55272427	C	T	51.46	10.03	0.32
GLOSSY15	9	55273108	C	G	57.29	10.76	0.36
GLOSSY15	9	58847231	C	T	22.12	5.12	0.17
GLOSSY15	9	59158465	T	C	41.58	8.71	0.24
GLOSSY15	9	59329989	A	G	37.91	8.02	0.24
GLOSSY15	9	59330005	C	T	48.09	9.52	0.31
GLOSSY15	9	64411557	C	T	35.21	7.60	0.21
GLOSSY15	9	64623367	A	C	49.29	9.66	0.32
GLOSSY15	9	64623380	G	C	32.46	6.99	0.23
GLOSSY15	9	64623611	C	G	19.85	4.71	0.15
GLOSSY15	9	73014627	G	A	32.57	7.11	0.20
GLOSSY15	9	73014628	C	G	40.55	8.44	0.25
GLOSSY15	9	73014629	A	C	32.57	7.11	0.20
GLOSSY15	9	110070816	C	G	20.39	4.85	0.14
GLOSSY15	9	136697586	G	T	25.54	5.69	0.21

Table A.2. Linkage map constructed on IPSRI population (n=133) using high confidence SNPs (0.43 < MAF <= 0.5, <0.1 missing data, and fixed for different alleles between inbreds IHP1 and ILP1) and candidate gene markers. Locus, chromosome (Chr), basepair position (BPPos), and Haldane cM map position (cM_Haldane) are indicated. Gaps in the linkage map are indicated by values of one in the column labeled Gap. cM distances across gaps were estimated by dividing the difference in basepairs between the two markers flanking the gap by the average basepairs per cM, as reported on a per chromosome basis in Table 3.5.

Locus	Chr	BPPos	Gap	cM_Haldane
S1_2957286	1	2957286	0	7.92
S1_2741561	1	2741561	0	8.76
S1_2753420	1	2753420	0	9.05
S1_2740925	1	2740925	0	9.27
S1_2753327	1	2753327	0	9.52
S1_2740913	1	2740913	0	9.78
S1_2753153	1	2753153	0	9.78
S1_3000850	1	3000850	0	11.31
S1_3279302	1	3279302	0	17.83
S1_3409322	1	3409322	0	20.63
S1_3409290	1	3409290	0	20.96
S1_3365546	1	3365546	0	21.67
S1_3439763	1	3439763	1	26.01
S1_6201190	1	6201190	1	33.28
S1_6412741	1	6412741	0	33.45
S1_6921987	1	6921987	0	38.08
S1_7390758	1	7390758	0	41.52
S1_7391420	1	7391420	0	41.93
S1_7390776	1	7390776	0	41.93
S1_7198081	1	7198081	0	42.44
S1_7401045	1	7401045	0	42.96
S1_7401017	1	7401017	0	42.96
S1_7401069	1	7401069	0	43.30
S1_7397142	1	7397142	0	43.55
S1_7389930	1	7389930	0	43.98
S1_7391404	1	7391404	0	44.50
S1_7389991	1	7389991	0	44.50
S1_7714442	1	7714442	0	48.97
S1_8113213	1	8113213	0	53.26
S1_8975828	1	8975828	0	56.77
S1_9396316	1	9396316	0	59.68
S1_9396639	1	9396639	0	59.93
S1_8515338	1	8515338	0	61.96
S1_8538881	1	8538881	0	62.45
S1_8637190	1	8637190	0	65.52
S1_8885156	1	8885156	0	66.77
S1_8882584	1	8882584	0	67.47
S1_8882564	1	8882564	0	67.55
S1_12312300	1	12312300	0	76.99
S1_12498912	1	12498912	0	79.03
S1_12344374	1	12344374	0	80.09
S1_12441345	1	12441345	0	80.10
S1_12344441	1	12344441	0	80.10
S1_12181197	1	12181197	0	80.35
S1_11945233	1	11945233	0	83.03
S1_11993149	1	11993149	0	86.34
S1_11242832	1	11242832	0	89.06
S1_12152579	1	12152579	0	91.97
S1_12150817	1	12150817	0	92.67
S1_12169708	1	12169708	0	93.27
S1_10960976	1	10960976	0	97.12
S1_10963206	1	10963206	0	97.30
S1_10899900	1	10899900	0	97.63
S1_10967130	1	10967130	0	97.63
S1_10902826	1	10902826	0	97.98
S1_10960811	1	10960811	0	98.75
S1_10970018	1	10970018	0	99.82
S1_10978004	1	10978004	0	100.79
S1_10978001	1	10978001	0	100.96
S1_10512623	1	10512623	0	106.20
S1_10512615	1	10512615	1	106.80
S1_17673689	1	17673689	1	125.66
S1_17673474	1	17673474	0	125.76
S1_17681208	1	17681208	0	126.91

Table A.2 continued.

S1_17679938	1	17679938	0	127.08
S1_17681173	1	17681173	0	127.17
S1_17681172	1	17681172	0	127.25
S1_17680181	1	17680181	0	127.33
S1_17625344	1	17625344	0	128.77
S1_17625350	1	17625350	0	128.77
S1_16783467	1	16783467	0	138.41
S1_16792461	1	16792461	0	138.58
S1_16627431	1	16627431	0	138.92
S1_16695375	1	16695375	0	139.01
S1_16698832	1	16698832	0	139.35
S1_15995448	1	15995448	0	141.14
S1_15995491	1	15995491	0	141.36
S1_15176108	1	15176108	0	144.10
S1_15353907	1	15353907	0	147.07
S1_15995473	1	15995473	0	148.03
S1_15997295	1	15997295	0	148.28
S1_16047143	1	16047143	0	148.94
S1_16950817	1	16950817	0	151.24
S1_17831094	1	17831094	0	154.12
S1_19100862	1	19100862	0	155.72
S1_19234051	1	19234051	0	155.90
S1_19642472	1	19642472	0	158.91
S1_19642337	1	19642337	0	158.99
S1_19979634	1	19979634	0	162.01
S1_21568640	1	21568640	0	162.69
S1_21473231	1	21473231	0	162.97
S1_21473288	1	21473288	0	163.15
S1_21543129	1	21543129	0	163.74
S1_20908061	1	20908061	0	164.26
S1_20698831	1	20698831	0	165.69
S1_23566233	1	23566233	1	171.58
S1_29787015	1	29787015	1	187.96
S1_29787054	1	29787054	0	188.14
S1_29156520	1	29156520	0	189.59
S1_28522579	1	28522579	0	191.46
S1_27303546	1	27303546	0	192.71
S1_27501695	1	27501695	0	193.14
S1_27395526	1	27395526	0	193.39
S1_27627317	1	27627317	0	194.16
S1_27627382	1	27627382	0	195.90
S1_27627301	1	27627301	0	195.90
S1_27626489	1	27626489	0	196.07
S1_27626514	1	27626514	0	196.07
S1_27626026	1	27626026	0	196.07
S1_27626051	1	27626051	0	196.07
S1_27626515	1	27626515	0	196.07
S1_27508151	1	27508151	0	196.56
S1_27524340	1	27524340	0	197.10
S1_27638765	1	27638765	0	197.53
S1_27172318	1	27172318	0	197.79
S1_27982375	1	27982375	0	198.57
S1_28293011	1	28293011	0	198.92
S1_28459269	1	28459269	0	199.09
S1_28643298	1	28643298	0	201.13
S1_28643335	1	28643335	0	201.30
S1_28961713	1	28961713	0	202.08
S1_29156625	1	29156625	0	202.60
S1_29156562	1	29156562	0	202.79
S1_29543320	1	29543320	0	203.64
S1_30042877	1	30042877	0	205.22
S1_30259081	1	30259081	0	205.39
S1_30279181	1	30279181	0	205.73
S1_29788926	1	29788926	1	210.27
S1_36918670	1	36918670	1	229.05
S1_36831913	1	36831913	0	229.43
S1_36831988	1	36831988	0	230.11
S1_36801883	1	36801883	0	230.89
S1_36973855	1	36973855	0	231.48
S1_37315846	1	37315846	0	231.84
S1_37842185	1	37842185	0	232.58
S1_37842905	1	37842905	0	232.78
S1_37132524	1	37132524	0	233.30
S1_38243957	1	38243957	0	233.90
S1_38130478	1	38130478	0	235.04
S1_37132704	1	37132704	0	235.75
S1_36918721	1	36918721	0	236.54
S1_36913868	1	36913868	0	237.14
S1_36913862	1	36913862	0	237.38
AsnS3	1	45114258	0	241.67
S1_44345174	1	44345174	0	242.46
S1_44346011	1	44346011	0	242.54
S1_44193311	1	44193311	0	242.71

Table A.2 continued.

S1_44343942	1	44343942	0	242.71
S1_44960114	1	44960114	0	242.71
S1_44960183	1	44960183	0	242.71
S1_44346036	1	44346036	0	242.79
S1_44959744	1	44959744	0	242.96
S1_44346024	1	44346024	0	243.22
S1_44346098	1	44346098	0	243.22
S1_44345120	1	44345120	0	243.31
S1_44373499	1	44373499	0	243.64
S1_46406150	1	46406150	0	244.89
S1_46406068	1	46406068	1	245.32
S1_52638614	1	52638614	1	261.74
S1_52642154	1	52642154	0	261.91
S1_52920283	1	52920283	0	263.45
S1_52920277	1	52920277	0	263.45
S1_52921116	1	52921116	0	263.45
S1_52161954	1	52161954	0	268.20
S1_51461771	1	51461771	1	271.60
S1_64718455	1	64718455	1	306.51
S1_65481985	1	65481985	0	307.57
S1_66387580	1	66387580	0	308.09
S1_65351132	1	65351132	0	308.61
S1_65485062	1	65485062	0	308.87
S1_65351115	1	65351115	0	308.95
S1_65427121	1	65427121	0	309.32
S1_68660614	1	68660614	0	314.31
S1_69287306	1	69287306	0	315.20
S1_68882477	1	68882477	0	315.37
S1_69865635	1	69865635	0	319.09
S1_69865634	1	69865634	0	319.26
S1_70769062	1	70769062	0	323.76
S1_71607318	1	71607318	0	330.78
S1_71607329	1	71607329	1	331.15
S1_98098030	1	98098030	1	400.92
S1_98342459	1	98342459	0	401.00
S1_98325832	1	98325832	0	401.00
S1_98762289	1	98762289	0	404.69
S1_98762298	1	98762298	0	405.09
S1_116265677	1	116265677	0	409.42
S1_116047729	1	116047729	0	409.68
S1_113530882	1	113530882	0	409.76
S1_116047759	1	116047759	0	409.85
S1_113530198	1	113530198	0	410.19
S1_116265903	1	116265903	0	410.64
S1_116265895	1	116265895	0	410.93
S1_113530921	1	113530921	0	411.81
S1_113530915	1	113530915	0	411.90
S1_116265779	1	116265779	0	412.08
S1_116266650	1	116266650	0	412.58
S1_119655679	1	119655679	0	413.71
S1_118261775	1	118261775	0	414.06
S1_119144429	1	119144429	0	414.40
S1_119412581	1	119412581	0	414.74
S1_118164463	1	118164463	0	415.34
S1_119377036	1	119377036	0	415.78
S1_119429834	1	119429834	0	416.06
S1_119378120	1	119378120	0	416.39
S1_119551660	1	119551660	0	416.73
S1_117703049	1	117703049	0	416.90
S1_118164394	1	118164394	0	416.98
S1_118164417	1	118164417	0	417.07
S1_118054462	1	118054462	0	417.20
S1_119136900	1	119136900	0	417.50
S1_118365564	1	118365564	0	417.60
S1_122471784	1	122471784	0	418.10
S1_121520628	1	121520628	0	418.42
S1_121545002	1	121545002	0	418.60
S1_122756867	1	122756867	0	418.77
S1_140888417	1	140888417	0	419.47
S1_140985391	1	140985391	0	420.16
S1_141076934	1	141076934	0	420.16
S1_139791469	1	139791469	0	420.33
S1_139790897	1	139790897	0	420.93
S1_139791465	1	139791465	0	421.02
S1_124126862	1	124126862	0	421.33
S1_121544970	1	121544970	0	421.41
S1_120364442	1	120364442	0	421.54
S1_121493056	1	121493056	0	421.63
S1_123767580	1	123767580	0	421.86
S1_121656103	1	121656103	0	422.09
S1_121493120	1	121493120	0	422.20
S1_122474698	1	122474698	0	422.71
S1_121653354	1	121653354	0	422.71

Table A.2 continued.

S1_121800570	1	121800570	0	422.90
S1_147891886	1	147891886	0	425.89
S1_147724658	1	147724658	0	426.29
S1_147979837	1	147979837	0	426.40
S1_147896068	1	147896068	1	426.49
S1_180339447	1	180339447	1	491.58
S1_190559456	1	190559456	0	499.14
S1_190558194	1	190558194	0	499.40
S1_189400889	1	189400889	0	500.55
S1_188088988	1	188088988	0	504.40
S1_188065174	1	188065174	0	504.83
S1_188034223	1	188034223	0	505.17
S1_187588875	1	187588875	0	512.51
S1_187646403	1	187646403	0	514.97
S1_187646720	1	187646720	0	515.14
S1_187885117	1	187885117	0	517.69
S1_188185464	1	188185464	0	522.05
S1_188904214	1	188904214	0	523.21
S1_188983541	1	188983541	0	523.29
S1_189688511	1	189688511	0	524.13
S1_192407739	1	192407739	0	527.57
S1_193600138	1	193600138	0	527.82
S1_193600186	1	193600186	0	527.86
S1_193592153	1	193592153	0	529.84
S1_194675064	1	194675064	0	532.25
S1_194676079	1	194676079	0	532.62
S1_194675174	1	194675174	0	532.93
S1_194763024	1	194763024	0	533.36
S1_194994383	1	194994383	0	534.05
S1_194993115	1	194993115	0	534.39
S1_194993075	1	194993075	0	534.39
S1_195139570	1	195139570	0	534.62
S1_195139655	1	195139655	0	534.99
S1_195139638	1	195139638	0	535.41
S1_195139593	1	195139593	0	535.75
S1_195331696	1	195331696	0	536.45
S1_195185966	1	195185966	0	536.71
S1_195331603	1	195331603	0	536.88
S1_195338409	1	195338409	0	537.21
S1_195558014	1	195558014	0	537.55
S1_195753586	1	195753586	0	538.80
S1_195723208	1	195723208	0	538.99
S1_195722459	1	195722459	0	541.12
S1_195722434	1	195722434	0	541.12
S1_196534684	1	196534684	0	544.37
S1_195878433	1	195878433	0	549.51
S1_195878426	1	195878426	0	549.78
S1_197751167	1	197751167	0	557.82
S1_197751140	1	197751140	0	557.91
S1_197748546	1	197748546	1	558.26
S1_198541547	1	198541547	1	560.34
S1_199963819	1	199963819	0	568.93
S1_200912858	1	200912858	0	578.70
S1_202160421	1	202160421	0	580.64
S1_201740357	1	201740357	0	580.83
S1_201739917	1	201739917	0	581.21
S1_202603667	1	202603667	0	582.47
S1_202298530	1	202298530	0	582.64
S1_202160385	1	202160385	0	583.79
S1_202160398	1	202160398	0	583.88
S1_202168404	1	202168404	0	584.63
S1_202168407	1	202168407	0	584.89
S1_202168472	1	202168472	0	585.17
S1_202160254	1	202160254	0	585.84
S1_200412107	1	200412107	0	592.92
S1_200413215	1	200413215	0	593.93
S1_205247369	1	205247369	1	603.68
S1_216581201	1	216581201	1	633.53
S1_215610681	1	215610681	0	634.46
S1_215610682	1	215610682	0	634.65
S1_215443933	1	215443933	0	635.68
S1_215443085	1	215443085	0	636.19
S1_215443935	1	215443935	0	636.45
S1_215443922	1	215443922	0	636.68
S1_215444048	1	215444048	0	636.87
S1_216581102	1	216581102	0	637.69
S1_216684777	1	216684777	0	637.90
S1_216684741	1	216684741	0	637.98
S1_216761867	1	216761867	0	638.77
S1_210096708	1	210096708	0	638.77
S1_211331330	1	211331330	0	645.76
S1_211199881	1	211199881	0	650.65
S1_211257805	1	211257805	0	651.44

Table A.2 continued.

S1_213153225	1	213153225	0	656.02
S1_212579132	1	212579132	0	657.34
S1_212573548	1	212573548	0	658.26
S1_211525444	1	211525444	0	660.06
S1_211523245	1	211523245	0	660.58
S1_223826032	1	223826032	0	670.51
S1_223826179	1	223826179	0	671.58
S1_223688064	1	223688064	0	676.88
S1_223313717	1	223313717	0	682.69
S1_222205772	1	222205772	0	686.92
S1_222547641	1	222547641	0	689.94
S1_222550310	1	222550310	0	690.02
S1_222547628	1	222547628	1	690.11
S1_233457728	1	233457728	1	718.85
S1_233457700	1	233457700	0	719.19
S1_234254504	1	234254504	0	724.35
S1_234221885	1	234221885	0	724.64
S1_234254741	1	234254741	0	725.04
S1_234258359	1	234258359	0	725.46
S1_234260766	1	234260766	0	725.63
S1_234260851	1	234260851	0	725.83
S1_234965905	1	234965905	0	726.06
S1_234965914	1	234965914	0	726.14
S1_235441603	1	235441603	0	726.31
S1_235365775	1	235365775	1	726.48
S1_239297667	1	239297667	1	736.84
S1_239297657	1	239297657	0	737.10
S1_239297642	1	239297642	0	737.43
S1_239534432	1	239534432	0	737.92
S1_239208777	1	239208777	0	738.36
S1_260956682	1	260956682	0	756.06
S1_261412168	1	261412168	0	757.70
S1_260959421	1	260959421	0	758.12
S1_257214217	1	257214217	0	768.22
S1_257214234	1	257214234	0	768.65
S1_256663874	1	256663874	0	770.58
S1_256663863	1	256663863	0	770.58
S1_254401049	1	254401049	0	774.01
S1_253798682	1	253798682	0	779.69
S1_253798663	1	253798663	0	779.90
S1_253798667	1	253798667	0	780.31
S1_253798755	1	253798755	0	780.65
S1_253798778	1	253798778	0	780.90
S1_253012113	1	253012113	0	791.90
S1_252632642	1	252632642	0	793.15
S1_252632654	1	252632654	0	793.49
S1_252631376	1	252631376	0	794.19
S1_240765377	1	240765377	0	810.65
S1_240766848	1	240766848	0	811.10
S1_240495001	1	240495001	0	811.27
S1_240765346	1	240765346	0	811.44
S1_240765379	1	240765379	0	811.53
S1_240495058	1	240495058	0	811.61
S1_241480712	1	241480712	0	815.21
S1_241480694	1	241480694	0	815.21
S1_241219259	1	241219259	0	815.98
S1_241261378	1	241261378	0	819.03
S1_243219401	1	243219401	0	822.63
S1_241508722	1	241508722	0	824.46
S1_250923454	1	250923454	0	830.80
S1_250913958	1	250913958	0	831.26
S1_251103140	1	251103140	0	831.58
S1_251039891	1	251039891	0	831.86
S1_251112677	1	251112677	0	831.95
S1_251995265	1	251995265	0	835.79
S1_242501726	1	242501726	0	842.97
S1_242502707	1	242502707	0	843.57
S1_242708951	1	242708951	0	843.66
S1_242581063	1	242581063	0	844.00
S1_242581078	1	242581078	0	844.09
S1_241481541	1	241481541	0	845.72
S1_241481582	1	241481582	0	845.89
S1_242501731	1	242501731	0	847.04
S1_253607742	1	253607742	0	891.33
S1_253570834	1	253570834	0	893.28
S1_253570835	1	253570835	1	893.39
S1_262322085	1	262322085		916.44
S1_262456979	1	262456979	0	916.92
S1_262459657	1	262459657	0	917.28
S1_262175904	1	262175904	0	917.64
S1_262183962	1	262183962	0	917.82
S1_263603503	1	263603503	0	921.18
S1_263752961	1	263752961	0	921.96

Table A.2 continued.

S1_264529819	1	264529819	0	922.57
S1_271191828	1	271191828	0	933.25
S1_271191547	1	271191547	0	933.43
S1_271191615	1	271191615	0	933.67
S1_271110124	1	271110124	0	934.26
S1_271982234	1	271982234	0	935.10
S1_273101305	1	273101305	0	936.66
S1_274424184	1	274424184	0	937.92
S1_274982683	1	274982683	0	938.99
S1_275647011	1	275647011	0	941.14
S1_275694088	1	275694088	0	941.39
S1_275693645	1	275693645	0	941.84
S1_275263332	1	275263332	0	942.77
S1_275033578	1	275033578	0	943.60
S1_274881483	1	274881483	0	946.28
S1_274881532	1	274881532	0	946.63
S1_274881534	1	274881534	0	946.98
S1_274723740	1	274723740	0	947.59
S1_274718673	1	274718673	0	947.76
S1_274718694	1	274718694	0	947.77
S1_274408175	1	274408175	0	948.63
S1_274408145	1	274408145	0	948.63
S1_273693058	1	273693058	0	949.32
S1_273445809	1	273445809	0	949.92
S1_273704126	1	273704126	0	950.54
S1_273696827	1	273696827	0	951.14
S1_273445797	1	273445797	0	951.14
S1_273233339	1	273233339	0	951.73
S1_272352985	1	272352985	0	952.09
S1_272940164	1	272940164	0	952.51
S1_272830287	1	272830287	0	952.94
S1_272832811	1	272832811	0	953.11
S1_272837440	1	272837440	0	953.28
S1_272830294	1	272830294	0	953.58
S1_270024525	1	270024525	0	954.77
S1_270671170	1	270671170	0	955.71
S1_270703043	1	270703043	0	956.17
S1_270703042	1	270703042	0	956.28
S1_270961887	1	270961887	0	956.62
S1_269643198	1	269643198	0	956.87
S1_270031325	1	270031325	0	956.95
S1_270024581	1	270024581	0	957.15
S1_269304433	1	269304433	0	959.36
S1_269298110	1	269298110	0	959.66
S1_269302116	1	269302116	0	959.96
S1_269298077	1	269298077	0	960.08
S1_268255455	1	268255455	0	962.68
S1_268308762	1	268308762	0	962.78
S1_268731509	1	268731509	0	964.49
S1_268740466	1	268740466	0	965.69
S1_269038066	1	269038066	0	967.05
S1_269038073	1	269038073	0	968.86
S1_268564469	1	268564469	0	970.25
S1_268955504	1	268955504	0	971.78
S1_268937377	1	268937377	0	972.16
S1_265680816	1	265680816	0	974.20
S1_266095785	1	266095785	0	975.17
S1_267783894	1	267783894	0	975.87
S1_267783859	1	267783859	0	975.95
S1_270982689	1	270982689	0	977.70
S1_271001512	1	271001512	0	979.46
S1_271001564	1	271001564	0	980.04
S1_271122548	1	271122548	0	983.09
S1_271122567	1	271122567	0	983.32
S1_276309892	1	276309892	0	986.96
S1_276119307	1	276119307	0	987.47
S1_275984187	1	275984187	0	988.82
S1_276324400	1	276324400	0	989.08
S1_276324391	1	276324391	0	989.24
S1_276325774	1	276325774	0	989.41
S1_276987537	1	276987537	0	990.38
S1_276995964	1	276995964	0	990.99
S1_277845026	1	277845026	0	991.50
S1_277844338	1	277844338	0	992.02
S1_277598415	1	277598415	0	992.10
S1_277846443	1	277846443	0	992.61
S1_277846455	1	277846455	0	992.62
S1_277348271	1	277348271	0	992.79
S1_277348267	1	277348267	0	992.87
S1_277310837	1	277310837	0	993.04
S1_278034349	1	278034349	0	993.47
S1_278034772	1	278034772	0	993.47
S1_278034277	1	278034277	0	993.47

Table A.2 continued.

S1_278047666	1	278047666	0	994.43
S1_278451739	1	278451739	0	996.05
S1_278604713	1	278604713	0	999.27
S1_278516224	1	278516224	0	999.54
S1_278604666	1	278604666	0	1000.04
S1_279187360	1	279187360	0	1000.42
S1_279124676	1	279124676	0	1000.67
S1_278513854	1	278513854	0	1001.64
S1_278650072	1	278650072	0	1002.33
S1_280344385	1	280344385	0	1006.62
S1_280579301	1	280579301	0	1008.12
S1_280578672	1	280578672	0	1008.55
S1_280579378	1	280579378	0	1008.63
S1_280579684	1	280579684	0	1008.75
S1_280579678	1	280579678	0	1008.97
S1_280581187	1	280581187	0	1009.51
S1_280219279	1	280219279	0	1010.18
S1_280379001	1	280379001	0	1010.61
S1_280579302	1	280579302	0	1010.78
S1_280685915	1	280685915	0	1011.02
S1_280579661	1	280579661	0	1011.19
S1_280581648	1	280581648	0	1011.30
S1_280826407	1	280826407	0	1012.91
S1_280826332	1	280826332	0	1014.72
S1_281071509	1	281071509	0	1015.09
S1_281071314	1	281071314	0	1015.21
S1_281071419	1	281071419	0	1016.50
S1_281168865	1	281168865	0	1018.31
S1_281168774	1	281168774	0	1019.18
S1_281168851	1	281168851	0	1019.51
S1_281168868	1	281168868	0	1019.78
S1_281633519	1	281633519	0	1021.96
S1_281632544	1	281632544	0	1023.90
S1_281898716	1	281898716	0	1024.70
S1_281898674	1	281898674	0	1025.31
S1_281889087	1	281889087	0	1025.40
S1_281910294	1	281910294	0	1025.83
S1_282471414	1	282471414	0	1027.34
S1_282528611	1	282528611	0	1027.73
S1_282529023	1	282529023	0	1027.99
S1_282530820	1	282530820	0	1028.24
S1_282745512	1	282745512	0	1028.50
S1_283431481	1	283431481	0	1028.66
S1_283196460	1	283196460	0	1029.00
S1_283253302	1	283253302	0	1029.26
S1_282745025	1	282745025	0	1029.43
S1_282745514	1	282745514	0	1029.51
S1_285077081	1	285077081	0	1030.97
S1_285069392	1	285069392	0	1031.23
S1_285096207	1	285096207	0	1031.96
S1_285078491	1	285078491	0	1032.65
S1_285528969	1	285528969	0	1034.82
S1_286182541	1	286182541	0	1035.74
S1_285982134	1	285982134	0	1036.07
S1_285982051	1	285982051	0	1036.27
S1_285978590	1	285978590	0	1038.11
S1_285970729	1	285970729	0	1038.19
S1_285785179	1	285785179	0	1038.91
S1_285340293	1	285340293	0	1040.83
S1_285971335	1	285971335	0	1042.47
S1_285965825	1	285965825	0	1042.72
S1_285970810	1	285970810	0	1043.15
S1_286406517	1	286406517	0	1043.76
S1_286406009	1	286406009	0	1043.92
S1_286439874	1	286439874	0	1044.18
S1_286439897	1	286439897	0	1044.50
S1_288013070	1	288013070	0	1050.23
S1_288013115	1	288013115	0	1050.84
S1_286400085	1	286400085	0	1057.24
S1_286182515	1	286182515	0	1057.79
S1_286981718	1	286981718	0	1060.51
S1_287065114	1	287065114	0	1061.05
S1_287722738	1	287722738	0	1061.48
S1_287474492	1	287474492	0	1061.60
S1_287704949	1	287704949	0	1061.73
S1_287728702	1	287728702	0	1061.89
S1_287474543	1	287474543	0	1062.18
S1_287704664	1	287704664	0	1062.29
S1_288396383	1	288396383	0	1063.83
S1_288396354	1	288396354	0	1063.83
S1_288397813	1	288397813	0	1064.80
S1_288397840	1	288397840	0	1065.19
S1_288863318	1	288863318	0	1068.76

Table A.2 continued.

S1_289598893	1	289598893	0	1070.11
S1_290286465	1	290286465	0	1071.75
S1_290204610	1	290204610	0	1072.09
S1_290204585	1	290204585	0	1072.18
S1_290296240	1	290296240	0	1072.88
S1_290268662	1	290268662	0	1072.88
S1_290231367	1	290231367	0	1073.41
S1_290286477	1	290286477	0	1073.92
S1_290286502	1	290286502	0	1074.00
S1_290286497	1	290286497	0	1074.26
S1_290306138	1	290306138	0	1075.50
S1_290306148	1	290306148	0	1075.93
S1_290957445	1	290957445	0	1076.60
S1_290957449	1	290957449	0	1076.84
S1_290955894	1	290955894	0	1077.34
S1_290957550	1	290957550	0	1077.72
S1_291402143	1	291402143	0	1078.23
S1_291726780	1	291726780	0	1079.77
S1_291865904	1	291865904	0	1080.37
S1_292223777	1	292223777	0	1082.43
S1_292087586	1	292087586	0	1082.69
S1_292090109	1	292090109	0	1083.10
S1_292403115	1	292403115	0	1083.74
S1_292518324	1	292518324	0	1085.25
S1_292577920	1	292577920	0	1086.17
S1_293627767	1	293627767	0	1090.76
S1_293944806	1	293944806	0	1092.18
S1_293942445	1	293942445	0	1092.28
S1_293797072	1	293797072	0	1092.98
S1_294085083	1	294085083	0	1093.95
S1_295020549	1	295020549	0	1095.00
S1_295066313	1	295066313	0	1095.26
S1_294135467	1	294135467	0	1095.61
S1_294097769	1	294097769	0	1095.67
S1_294044189	1	294044189	0	1096.05
S1_294905180	1	294905180	0	1097.54
S1_295316364	1	295316364	0	1101.58
S1_295309095	1	295309095	0	1101.89
S1_295309110	1	295309110	0	1102.25
S1_295349373	1	295349373	0	1103.50
S1_295439172	1	295439172	0	1104.46
S1_295548488	1	295548488	0	1104.72
S1_295630719	1	295630719	0	1106.18
S1_295596748	1	295596748	0	1106.50
S1_295475829	1	295475829	0	1106.50
S1_295596727	1	295596727	0	1106.67
S1_295349423	1	295349423	0	1107.10
S1_295596779	1	295596779	0	1107.79
S1_295588843	1	295588843	0	1107.96
S1_295762684	1	295762684	0	1108.75
S1_296769016	1	296769016	0	1111.54
S1_296768174	1	296768174	0	1111.62
S1_296710295	1	296710295	0	1112.23
S1_296710299	1	296710299	0	1112.57
S1_296771036	1	296771036	0	1113.32
S1_296891397	1	296891397	0	1115.94
S1_297977885	1	297977885	0	1120.07
S1_298411038	1	298411038	0	1123.11
S1_298687882	1	298687882	0	1123.99
S1_298824042	1	298824042	0	1124.25
S1_298983558	1	298983558	0	1125.87
S1_299199101	1	299199101	0	1126.04
S1_299196965	1	299196965	0	1126.36
S1_299199071	1	299199071	0	1126.36
S1_299094505	1	299094505	0	1126.84
S1_299122093	1	299122093	0	1127.29
S1_299462225	1	299462225	0	1128.42
S1_299389006	1	299389006	0	1128.62
S1_299416524	1	299416524	0	1128.72
S1_299416499	1	299416499	0	1128.86
S1_299468209	1	299468209	0	1129.18
S1_299468223	1	299468223	0	1129.47
S1_299405479	1	299405479	0	1129.75
S1_300037709	1	300037709	0	1131.67
S1_299609421	1	299609421	1	1132.52
S2_10208	2	10208	1	0.02
S2_847881	2	847881	0	0.74
S2_841463	2	841463	0	0.95
S2_1289375	2	1289375	0	3.05
S2_1270245	2	1270245	0	3.90
S2_1040723	2	1040723	0	4.43
S2_1035863	2	1035863	0	4.62
S2_1463153	2	1463153	0	5.32

Table A.2 continued.

S2_1437975	2	1437975	0	5.66
S2_1448460	2	1448460	0	6.28
S2_1629862	2	1629862	0	8.34
S2_1683494	2	1683494	0	9.23
S2_1634173	2	1634173	0	9.32
S2_1632775	2	1632775	0	12.00
S2_1974401	2	1974401	0	16.63
S2_2051889	2	2051889	1	18.46
S2_2485994	2	2485994	1	19.22
S2_2493148	2	2493148	0	20.38
S2_3417138	2	3417138	0	25.97
S2_3417223	2	3417223	0	26.14
S2_3417221	2	3417221	0	26.74
S2_3667969	2	3667969	0	30.71
S2_3795378	2	3795378	0	30.88
S2_3795385	2	3795385	0	31.24
S2_3749198	2	3749198	0	31.72
S2_3974601	2	3974601	0	33.09
S2_4162830	2	4162830	0	34.31
S2_4162842	2	4162842	0	34.99
S2_4052749	2	4052749	0	35.25
S2_3875397	2	3875397	0	36.65
S2_3974629	2	3974629	0	37.69
S2_4465539	2	4465539	0	43.58
S2_4466356	2	4466356	0	43.65
S2_4580310	2	4580310	0	44.15
S2_4626286	2	4626286	0	45.24
S2_4580446	2	4580446	0	48.35
S2_4889615	2	4889615	0	49.94
S2_4886578	2	4886578	0	50.28
ASNase3UTR	2	5073171	0	51.06
S2_5763550	2	5763550	0	56.67
S2_6597036	2	6597036	0	59.26
S2_6721246	2	6721246	0	61.62
S2_6823195	2	6823195	0	63.36
S2_6849919	2	6849919	0	63.36
S2_6850493	2	6850493	0	63.36
S2_6849949	2	6849949	0	63.36
S2_6849658	2	6849658	0	63.53
S2_6850397	2	6850397	0	63.84
S2_6849912	2	6849912	0	67.50
S2_7047102	2	7047102	0	68.07
S2_6980493	2	6980493	0	68.58
S2_6979676	2	6979676	0	68.92
S2_6975163	2	6975163	0	69.09
S2_6973574	2	6973574	0	69.35
S2_6979796	2	6979796	0	69.43
S2_7178335	2	7178335	0	70.24
S2_7095185	2	7095185	0	70.73
S2_6983601	2	6983601	0	71.61
S2_6991246	2	6991246	0	72.13
S2_6982110	2	6982110	0	72.82
S2_7001231	2	7001231	0	73.25
S2_6973575	2	6973575	0	73.42
S2_7182424	2	7182424	0	74.48
S2_7182504	2	7182504	0	74.48
S2_7209888	2	7209888	0	74.65
S2_7196568	2	7196568	0	74.65
S2_7292319	2	7292319	0	75.25
S2_7190513	2	7190513	0	76.67
S2_7190543	2	7190543	0	76.77
S2_7196295	2	7196295	0	77.03
S2_7209893	2	7209893	0	77.20
S2_7209908	2	7209908	0	77.28
S2_7289634	2	7289634	0	77.76
S2_7220412	2	7220412	0	77.93
S2_7220403	2	7220403	0	78.02
S2_7292370	2	7292370	0	78.27
S2_7309497	2	7309497	0	78.27
S2_8287296	2	8287296	0	80.20
S2_7958992	2	7958992	0	80.25
S2_7959762	2	7959762	0	80.46
S2_7953284	2	7953284	0	80.87
S2_8781697	2	8781697	0	80.95
S2_7951136	2	7951136	0	81.55
S2_7951052	2	7951052	0	81.81
S2_7950068	2	7950068	0	81.98
S2_7959907	2	7959907	0	82.43
S2_8289103	2	8289103	0	83.01
S2_8287267	2	8287267	0	83.13
S2_8808694	2	8808694	0	83.88
S2_8808696	2	8808696	0	84.06
S2_8808714	2	8808714	0	84.68

Table A.2 continued.

S2_8808660	2	8808660	0	84.76
S2_8809102	2	8809102	0	85.46
S2_8890211	2	8890211	0	86.45
S2_8872194	2	8872194	0	87.17
S2_8809007	2	8809007	0	87.77
S2_8808624	2	8808624	0	87.86
S2_8808641	2	8808641	0	88.46
S2_8915262	2	8915262	0	89.63
S2_8919844	2	8919844	0	90.24
S2_8897645	2	8897645	0	91.00
S2_8890223	2	8890223	0	91.10
S2_8809535	2	8809535	0	91.44
S2_8809710	2	8809710	0	91.76
S2_8809708	2	8809708	0	91.91
S2_8996826	2	8996826	0	93.83
S2_8996844	2	8996844	0	94.83
S2_8996601	2	8996601	0	94.83
S2_9000336	2	9000336	0	95.12
S2_9000321	2	9000321	0	95.31
S2_9006121	2	9006121	0	95.63
S2_8996922	2	8996922	0	95.92
S2_8996934	2	8996934	0	96.05
S2_9266113	2	9266113	0	97.11
S2_9267177	2	9267177	0	97.19
S2_9316693	2	9316693	0	97.63
S2_9318468	2	9318468	0	100.20
S2_9131012	2	9131012	0	102.18
S2_9557094	2	9557094	0	105.08
S2_9944903	2	9944903	0	105.86
S2_9561501	2	9561501	0	106.56
S2_9557062	2	9557062	0	106.86
S2_10497832	2	10497832	0	118.53
S2_10553685	2	10553685	0	126.92
S2_11546639	2	11546639	0	129.46
S2_11582554	2	11582554	0	129.98
S2_12016517	2	12016517	0	134.71
S2_12013585	2	12013585	0	134.89
S2_12255052	2	12255052	0	139.65
S2_12268170	2	12268170	0	140.44
S2_12632197	2	12632197	0	142.46
S2_12643753	2	12643753	0	144.13
S2_12686051	2	12686051	0	144.80
S2_12898836	2	12898836	0	145.51
S2_12921336	2	12921336	0	145.76
S2_12921363	2	12921363	0	145.85
S2_12802632	2	12802632	0	146.28
S2_12797703	2	12797703	0	146.65
S2_14267092	2	14267092	0	150.86
S2_14267094	2	14267094	0	151.11
S2_14267377	2	14267377	0	151.38
S2_14804644	2	14804644	0	151.80
S2_15013069	2	15013069	0	152.15
S2_15013191	2	15013191	0	152.15
S2_15243278	2	15243278	0	153.03
S2_15342050	2	15342050	0	153.60
S2_15341213	2	15341213	0	154.02
S2_15283638	2	15283638	0	154.38
S2_15340166	2	15340166	0	155.05
S2_15909179	2	15909179	0	155.88
S2_15495809	2	15495809	0	156.74
S2_15682750	2	15682750	0	157.17
S2_15482198	2	15482198	0	157.36
S2_15472398	2	15472398	0	157.57
S2_15141036	2	15141036	0	157.96
S2_15247020	2	15247020	0	157.96
S2_15141107	2	15141107	0	157.96
S2_18621068	2	18621068	0	164.30
S2_19195332	2	19195332	0	165.36
S2_19258380	2	19258380	0	165.45
S2_19195358	2	19195358	0	165.60
S2_19262986	2	19262986	0	165.63
S2_19195357	2	19195357	0	165.65
S2_19195355	2	19195355	0	165.90
S2_19195353	2	19195353	0	166.15
S2_19195351	2	19195351	0	166.41
S2_18767008	2	18767008	0	166.93
S2_17544291	2	17544291	0	169.20
S2_17479298	2	17479298	0	169.48
S2_17477044	2	17477044	0	170.02
S2_17544284	2	17544284	0	170.11
S2_16123899	2	16123899	0	176.23
S2_16123872	2	16123872	0	176.40
S2_16306984	2	16306984	1	180.40

Table A.2 continued.

S2_17676757	2	17676757	1	182.80
S2_17792034	2	17792034	0	183.63
S2_17862255	2	17862255	0	183.91
S2_17792058	2	17792058	0	184.48
S2_17792024	2	17792024	0	184.59
S2_17792044	2	17792044	0	184.75
S2_17862395	2	17862395	1	185.04
S2_27546469	2	27546469	1	201.97
S2_26982536	2	26982536	0	202.32
S2_27000009	2	27000009	0	202.95
S2_27052009	2	27052009	0	203.05
S2_27052063	2	27052063	0	203.13
S2_27000068	2	27000068	0	203.30
S2_26881506	2	26881506	0	203.73
S2_26881468	2	26881468	0	203.98
S2_26881482	2	26881482	0	204.45
S2_27253339	2	27253339	0	205.06
S2_27502162	2	27502162	0	205.83
S2_27501544	2	27501544	0	206.28
S2_25387853	2	25387853	0	207.17
S2_26296866	2	26296866	0	207.44
S2_26435949	2	26435949	0	207.86
S2_26294073	2	26294073	0	207.94
S2_26294075	2	26294075	0	208.44
S2_26287935	2	26287935	0	208.85
S2_26717573	2	26717573	0	209.69
S2_28866436	2	28866436	0	215.84
S2_28866381	2	28866381	0	218.74
S2_29873994	2	29873994	0	227.63
S2_36318367	2	36318367	0	233.81
S2_36318442	2	36318442	0	234.15
S2_36318426	2	36318426	0	234.54
S2_34570841	2	34570841	0	237.74
S2_36452777	2	36452777	0	238.71
S2_38322804	2	38322804	0	242.54
S2_38140566	2	38140566	1	243.98
S2_43342618	2	43342618	1	253.07
S2_43379184	2	43379184	0	253.15
S2_43558519	2	43558519	0	253.39
S2_43353338	2	43353338	0	253.49
S2_43428492	2	43428492	0	253.79
S2_44699694	2	44699694	0	253.79
S2_44718835	2	44718835	0	255.52
S2_44699768	2	44699768	0	255.66
S2_44718853	2	44718853	0	255.88
S2_44719287	2	44719287	0	256.09
S2_44719321	2	44719321	0	256.17
S2_44698658	2	44698658	0	256.34
S2_44719259	2	44719259	0	256.51
S2_44719226	2	44719226	0	258.65
S2_44435558	2	44435558	1	261.22
S2_62740879	2	62740879	1	293.22
S2_62291361	2	62291361	0	293.49
S2_62291362	2	62291362	0	294.07
S2_62378375	2	62378375	0	294.29
S2_62291306	2	62291306	0	294.63
S2_62291321	2	62291321	0	294.72
S2_62291206	2	62291206	0	294.89
S2_62740952	2	62740952	0	295.06
S2_62740939	2	62740939	0	295.14
S2_70285749	2	70285749	0	300.30
S2_70285767	2	70285767	0	300.56
S2_113313890	2	113313890	0	307.64
S2_113315866	2	113315866	1	309.54
S2_199173727	2	199173727	1	459.62
S2_199175422	2	199175422	0	460.60
S2_200406183	2	200406183	0	464.11
S2_203945187	2	203945187	0	468.01
S2_203945244	2	203945244	0	468.78
S2_203633916	2	203633916	0	469.57
S2_203633895	2	203633895	0	469.65
S2_203633871	2	203633871	0	469.97
S2_203257736	2	203257736	0	472.69
S2_203197017	2	203197017	0	477.81
S2_203063382	2	203063382	0	478.47
S2_203059599	2	203059599	0	479.03
S2_197146150	2	197146150	0	483.87
S2_197146145	2	197146145	0	484.26
S2_197146144	2	197146144	0	484.46
S2_196740101	2	196740101	0	485.74
S2_204882463	2	204882463	0	493.03
S2_206948325	2	206948325	0	495.39
S2_206896637	2	206896637	0	495.47

Table A.2 continued.

S2_207613202	2	207613202	0	496.92
S2_206624952	2	206624952	0	499.17
S2_206624854	2	206624854	0	500.23
S2_206624873	2	206624873	0	500.32
S2_206624902	2	206624902	0	500.63
S2_206701874	2	206701874	0	501.27
S2_199462332	2	199462332	0	509.88
S2_199459654	2	199459654	0	510.06
S2_199344380	2	199344380	0	510.16
S2_198723577	2	198723577	0	512.30
S2_198723586	2	198723586	0	513.70
S2_198394528	2	198394528	0	514.13
S2_198394491	2	198394491	0	514.13
S2_198404379	2	198404379	1	514.48
S2_208120431	2	208120431	1	531.46
S2_208612070	2	208612070	0	532.65
S2_208672697	2	208672697	0	532.82
S2_209131855	2	209131855	0	535.07
S2_209114287	2	209114287	0	535.46
S2_209109091	2	209109091	0	535.84
S2_209125351	2	209125351	0	536.44
S2_209110820	2	209110820	0	536.84
S2_209110675	2	209110675	1	537.21
S2_209537299	2	209537299	1	537.95
S2_209283642	2	209283642	0	538.54
S2_209283727	2	209283727	0	538.97
S2_209278340	2	209278340	0	539.13
S2_209278273	2	209278273	0	539.30
S2_209283539	2	209283539	0	539.30
S2_209283622	2	209283622	0	539.30
S2_209283591	2	209283591	0	539.41
S2_211723395	2	211723395	0	547.07
S2_211521319	2	211521319	0	547.54
S2_215040841	2	215040841	0	553.69
S2_211722498	2	211722498	0	559.56
S2_211722499	2	211722499	0	559.73
S2_218463283	2	218463283	0	568.23
S2_219021132	2	219021132	0	569.84
S2_217650053	2	217650053	0	570.90
S2_219020035	2	219020035	1	571.96
S2_220926291	2	220926291	1	575.29
S2_220980384	2	220980384	1	577.54
S2_223644255	2	223644255	1	582.19
S2_225126141	2	225126141	0	587.08
S2_224907504	2	224907504	0	587.92
S2_224467227	2	224467227	0	589.26
S2_225157881	2	225157881	0	590.42
S2_225157541	2	225157541	0	591.48
S2_225704017	2	225704017	0	594.50
S2_226203239	2	226203239	0	596.46
S2_226393054	2	226393054	0	599.03
S2_228859091	2	228859091	0	600.58
S2_228801647	2	228801647	0	601.28
S2_229287355	2	229287355	0	604.07
S2_229287384	2	229287384	1	604.24
S2_234259307	2	234259307	1	612.93
S2_234259389	2	234259389	0	613.10
S2_234259526	2	234259526	0	614.05
S2_236972431	2	236972431	0	618.93
S2_236966550	2	236966550	0	618.93
S2_236971705	2	236971705	1	618.93
S3_3694083	3	3694083	1	8.60
S3_3692916	3	3692916	0	9.82
S3_3559633	3	3559633	0	13.95
S3_3834594	3	3834594	0	16.12
S3_3832105	3	3832105	0	17.44
S3_4153027	3	4153027	0	18.16
S3_3924573	3	3924573	0	18.40
S3_3989171	3	3989171	0	18.49
S3_4148360	3	4148360	0	19.37
S3_4542492	3	4542492	0	23.16
S3_4862339	3	4862339	0	26.98
S3_4862374	3	4862374	0	27.08
S3_4887396	3	4887396	0	28.83
BNLG1144	3	5220112	0	32.56
S3_5671567	3	5671567	0	33.87
S3_5671559	3	5671559	0	33.98
S3_5777895	3	5777895	0	34.68
S3_5671585	3	5671585	0	35.42
S3_5429003	3	5429003	0	37.07
S3_5669203	3	5669203	0	37.58
S3_5581262	3	5581262	0	37.75
S3_5675783	3	5675783	0	37.75

Table A.2 continued.

S3_5578032	3	5578032	0	37.92
S3_5566750	3	5566750	0	38.07
S3_5566785	3	5566785	0	38.34
S3_5581196	3	5581196	0	38.44
S3_5885090	3	5885090	0	43.45
S3_5884892	3	5884892	0	43.64
S3_6294212	3	6294212	0	48.55
S3_6295479	3	6295479	0	49.04
S3_6294326	3	6294326	0	49.33
S3_6523471	3	6523471	0	51.08
S3_6523511	3	6523511	0	51.87
S3_6671078	3	6671078	0	53.62
S3_6671064	3	6671064	0	54.97
S3_7123327	3	7123327	0	59.86
S3_7524750	3	7524750	0	61.90
S3_7969639	3	7969639	0	63.70
S3_8208538	3	8208538	0	63.90
S3_8974483	3	8974483	0	65.64
S3_9039040	3	9039040	0	65.81
S3_9114622	3	9114622	0	66.26
S3_10140923	3	10140923	0	69.14
S3_10466314	3	10466314	0	72.64
S3_10469610	3	10469610	0	73.90
S3_10512183	3	10512183	0	74.80
S3_10512168	3	10512168	0	76.15
S3_10674683	3	10674683	1	79.70
S3_26309154	3	26309154	1	116.10
S3_26309157	3	26309157	0	116.28
S3_26309152	3	26309152	0	116.28
S3_20704745	3	20704745	0	126.14
S3_20426207	3	20426207	0	126.82
S3_20417461	3	20417461	0	127.00
S3_20417430	3	20417430	0	127.16
S3_20175121	3	20175121	0	127.68
S3_20175116	3	20175116	0	127.85
S3_19940304	3	19940304	0	128.72
S3_19945628	3	19945628	0	128.81
S3_19756027	3	19756027	0	129.50
S3_17829740	3	17829740	0	131.34
S3_17175578	3	17175578	0	132.68
S3_17398937	3	17398937	0	134.93
S3_17398949	3	17398949	0	134.93
S3_14930268	3	14930268	0	136.27
S3_14408461	3	14408461	0	136.28
S3_14408510	3	14408510	0	136.37
S3_15434794	3	15434794	0	136.71
S3_18003371	3	18003371	0	138.02
S3_19558134	3	19558134	0	138.61
S3_19924853	3	19924853	0	139.37
S3_20175108	3	20175108	0	139.74
S3_20742365	3	20742365	0	140.35
S3_20426181	3	20426181	0	140.60
S3_21260986	3	21260986	0	140.77
S3_20417478	3	20417478	0	141.43
S3_20417480	3	20417480	0	142.40
S3_21756457	3	21756457	0	145.39
S3_21756473	3	21756473	0	145.47
S3_21463409	3	21463409	0	145.73
S3_21429893	3	21429893	0	145.73
S3_21850388	3	21850388	0	146.15
S3_21855768	3	21855768	0	146.42
S3_21855790	3	21855790	0	146.53
S3_21563863	3	21563863	0	146.61
S3_21679984	3	21679984	0	146.70
S3_22888804	3	22888804	0	147.93
S3_22818931	3	22818931	0	148.09
S3_22738078	3	22738078	0	148.19
S3_22726480	3	22726480	0	149.14
S3_25340935	3	25340935	0	151.72
S3_25355371	3	25355371	0	152.33
S3_29613320	3	29613320	0	154.17
S3_31481725	3	31481725	0	154.96
S3_31454287	3	31454287	0	155.30
S3_29979146	3	29979146	0	155.86
S3_31806304	3	31806304	0	157.85
S3_31806288	3	31806288	0	157.98
S3_34497526	3	34497526	0	160.28
S3_34681473	3	34681473	0	160.83
S3_35502435	3	35502435	0	161.38
S3_35502438	3	35502438	0	161.58
S3_39212756	3	39212756	0	162.57
S3_39212788	3	39212788	0	162.75
S3_36316977	3	36316977	0	163.39

Table A.2 continued.

S3_80133478	3	80133478	0	169.34
S3_125158944	3	125158944	0	176.31
S3_124730549	3	124730549	0	177.51
S3_124730507	3	124730507	0	177.59
S3_124731476	3	124731476	0	178.04
S3_124731503	3	124731503	0	178.29
S3_125426610	3	125426610	0	179.13
S3_125426639	3	125426639	0	179.30
S3_123150854	3	123150854	0	182.84
S3_123267175	3	123267175	0	183.54
S3_122061563	3	122061563	0	184.85
S3_119638562	3	119638562	0	185.31
S3_119614021	3	119614021	0	185.46
S3_117255123	3	117255123	0	186.78
S3_118065768	3	118065768	0	187.33
S3_118065660	3	118065660	0	187.41
S3_118065766	3	118065766	0	187.49
S3_118065576	3	118065576	0	187.77
S3_118065765	3	118065765	0	188.18
S3_122110047	3	122110047	0	189.77
S3_122067315	3	122067315	0	190.33
S3_122058832	3	122058832	0	190.68
S3_86840519	3	86840519	0	192.82
S3_86840567	3	86840567	0	192.96
S3_117089716	3	117089716	0	193.39
S3_117087559	3	117087559	0	193.47
S3_114086391	3	114086391	0	193.90
S3_115292082	3	115292082	0	194.42
S3_86841029	3	86841029	0	194.85
S3_114792327	3	114792327	0	194.93
S3_85663005	3	85663005	0	195.90
S3_113425715	3	113425715	0	196.24
S3_86840502	3	86840502	0	196.49
S3_86840524	3	86840524	0	196.66
S3_85714955	3	85714955	0	197.26
S3_127853314	3	127853314	0	200.85
S3_127930789	3	127930789	0	201.60
S3_127930796	3	127930796	0	201.68
S3_128668374	3	128668374	0	202.20
S3_128668382	3	128668382	0	202.40
S3_128668383	3	128668383	0	202.79
S3_131330626	3	131330626	0	208.09
S3_131330627	3	131330627	1	208.53
S3_142730653	3	142730653	1	235.07
S3_143801262	3	143801262	0	236.42
S3_143697868	3	143697868	0	236.63
S3_145178610	3	145178610	0	238.29
S3_145178393	3	145178393	0	238.47
S3_145178377	3	145178377	0	238.48
S3_144813717	3	144813717	0	238.58
S3_145176210	3	145176210	0	239.19
S3_145481816	3	145481816	0	240.83
S3_145489637	3	145489637	0	241.35
S3_145476370	3	145476370	0	241.96
S3_145183490	3	145183490	0	243.81
S3_147292600	3	147292600	0	246.07
S3_146966730	3	146966730	0	246.32
S3_147411072	3	147411072	0	246.49
S3_147411909	3	147411909	0	246.66
S3_147292528	3	147292528	0	247.00
S3_147411957	3	147411957	0	247.35
S3_147408822	3	147408822	0	247.67
S3_147408820	3	147408820	0	247.78
S3_147407908	3	147407908	0	247.80
S3_147677571	3	147677571	0	249.76
S3_147677623	3	147677623	0	249.93
S3_147938363	3	147938363	0	250.36
S3_148300043	3	148300043	0	251.15
S3_148833327	3	148833327	0	251.23
S3_149243466	3	149243466	0	251.83
S3_149234794	3	149234794	0	252.17
S3_148395663	3	148395663	0	252.60
S3_148395711	3	148395711	0	252.68
S3_148797492	3	148797492	0	252.96
S3_148231562	3	148231562	0	253.72
S3_148231561	3	148231561	0	253.82
S3_148228297	3	148228297	0	256.19
S3_147938806	3	147938806	0	256.88
S3_152173669	3	152173669	0	259.62
S3_152056774	3	152056774	0	260.23
S3_152056755	3	152056755	0	260.40
S3_150171989	3	150171989	0	262.04
S3_150172218	3	150172218	0	262.29

Table A.2 continued.

S3_150170187	3	150170187	0	262.47
S3_150169329	3	150169329	0	262.64
S3_152498220	3	152498220	0	263.60
S3_153262849	3	153262849	0	263.69
S3_153262823	3	153262823	0	263.81
S3_152494431	3	152494431	0	263.86
S3_153262873	3	153262873	0	263.87
S3_153764021	3	153764021	0	264.13
S3_154373313	3	154373313	0	264.64
S3_153770222	3	153770222	0	264.75
S3_154563044	3	154563044	0	265.57
S3_154408488	3	154408488	0	266.75
S3_154373561	3	154373561	0	266.84
S3_154373563	3	154373563	0	266.84
S3_154076058	3	154076058	0	267.11
S3_154250438	3	154250438	0	267.58
S3_154562565	3	154562565	0	269.63
S3_154592839	3	154592839	0	269.98
S3_154592805	3	154592805	0	270.67
S3_154592811	3	154592811	0	270.87
S3_154076084	3	154076084	0	271.15
S3_154071852	3	154071852	0	271.33
S3_154071884	3	154071884	0	271.52
S3_154071843	3	154071843	0	271.74
S3_154071838	3	154071838	0	271.86
S3_154071841	3	154071841	0	272.07
S3_154124939	3	154124939	0	272.40
S3_154592916	3	154592916	0	273.15
S3_154592901	3	154592901	0	273.15
S3_154592919	3	154592919	0	273.32
S3_154710676	3	154710676	0	274.28
S3_154625214	3	154625214	0	275.05
S3_154661193	3	154661193	0	275.68
S3_154724691	3	154724691	0	275.83
S3_154659620	3	154659620	0	276.00
S3_154975362	3	154975362	0	276.95
S3_154975353	3	154975353	0	277.55
S3_154974554	3	154974554	0	278.57
S3_155318175	3	155318175	0	279.01
S3_156814134	3	156814134	0	279.68
S3_156814126	3	156814126	0	279.81
S3_156936844	3	156936844	0	280.01
S3_156964693	3	156964693	0	280.27
S3_156964651	3	156964651	0	280.52
S3_156634936	3	156634936	0	280.77
S3_159865108	3	159865108	0	284.50
S3_159865141	3	159865141	0	284.65
S3_160304559	3	160304559	0	285.43
S3_160087106	3	160087106	0	285.76
S3_161559514	3	161559514	0	286.62
S3_161574455	3	161574455	0	287.18
S3_161956024	3	161956024	0	287.75
S3_162079860	3	162079860	0	288.80
S3_162587502	3	162587502	0	290.13
S3_162602877	3	162602877	0	297.28
S3_162318060	3	162318060	0	298.63
S3_162702071	3	162702071	0	299.68
S3_162704555	3	162704555	0	299.71
S3_162702048	3	162702048	0	299.72
S3_162702024	3	162702024	0	299.97
S3_163654032	3	163654032	0	301.16
S3_164391477	3	164391477	0	306.02
S3_164173690	3	164173690	0	306.51
S3_164192755	3	164192755	0	307.80
S3_170459573	3	170459573	0	313.80
S3_170423229	3	170423229	0	315.42
S3_170423245	3	170423245	0	315.86
S3_170423197	3	170423197	0	316.12
S3_175937235	3	175937235	0	324.93
S3_175937257	3	175937257	0	325.36
S3_170865296	3	170865296	0	334.79
S3_170865293	3	170865293	0	334.88
S3_172050026	3	172050026	0	335.40
S3_171900685	3	171900685	0	335.48
S3_172048999	3	172048999	0	335.48
S3_172049025	3	172049025	0	335.65
S3_170865297	3	170865297	0	335.65
S3_171901917	3	171901917	0	335.65
S3_172336215	3	172336215	0	336.90
S3_172335957	3	172335957	0	336.98
S3_172332632	3	172332632	0	337.41
S3_172332527	3	172332527	0	337.41
S3_172198924	3	172198924	0	338.01

Table A.2 continued.

S3_172199020	3	172199020	0	338.44
S3_172132212	3	172132212	0	338.61
S3_172332520	3	172332520	0	338.61
S3_172372690	3	172372690	0	338.78
S3_172379630	3	172379630	0	338.84
S3_172407547	3	172407547	0	339.29
S3_172407530	3	172407530	0	339.29
S3_172407510	3	172407510	0	339.46
S3_173477021	3	173477021	0	340.61
S3_175869148	3	175869148	0	342.58
S3_175868965	3	175868965	0	342.58
S3_175999785	3	175999785	0	343.83
S3_176805947	3	176805947	0	344.62
S3_176805416	3	176805416	0	344.70
S3_176805405	3	176805405	0	344.87
S3_176805743	3	176805743	0	344.96
S3_176677912	3	176677912	0	345.41
S3_176805413	3	176805413	0	346.00
S3_177442272	3	177442272	0	348.13
S3_177445546	3	177445546	0	348.21
S3_177442227	3	177442227	0	348.29
S3_177445471	3	177445471	0	348.55
S3_178135987	3	178135987	0	353.43
S3_178135993	3	178135993	0	353.53
S3_179228490	3	179228490	0	356.24
S3_179994843	3	179994843	0	358.74
S3_179994880	3	179994880	0	359.17
S3_179791226	3	179791226	0	359.17
S3_179796726	3	179796726	0	359.34
S3_179803703	3	179803703	0	359.34
S3_179796715	3	179796715	0	359.34
S3_179803733	3	179803733	0	359.34
S3_179803722	3	179803722	0	359.42
S3_180509473	3	180509473	0	361.06
S3_180598426	3	180598426	0	361.58
S3_180598399	3	180598399	0	361.66
S3_180598368	3	180598368	0	362.36
S3_180598606	3	180598606	0	362.62
S3_180198931	3	180198931	0	363.22
S3_181279341	3	181279341	0	367.56
S3_181279379	3	181279379	0	367.82
S3_181815753	3	181815753	0	370.58
S3_181815705	3	181815705	0	371.13
S3_182046436	3	182046436	0	372.85
S3_181910732	3	181910732	0	374.12
S3_182044355	3	182044355	0	374.91
S3_182710243	3	182710243	0	379.05
S3_182127057	3	182127057	0	381.15
S3_182127012	3	182127012	0	381.36
S3_183638823	3	183638823	0	383.10
S3_183554000	3	183554000	0	384.84
S3_182589030	3	182589030	0	386.18
S3_182127055	3	182127055	0	386.36
S3_182127054	3	182127054	0	386.48
S3_183646761	3	183646761	0	388.21
S3_186779991	3	186779991	0	391.92
S3_186071755	3	186071755	0	395.42
S3_186210751	3	186210751	0	395.85
S3_186795782	3	186795782	0	397.00
S3_186383801	3	186383801	0	397.17
S3_186486750	3	186486750	0	397.26
S3_186722887	3	186722887	0	397.87
S3_187434911	3	187434911	0	400.66
S3_187438185	3	187438185	0	400.86
S3_187475607	3	187475607	0	401.09
S3_187484339	3	187484339	0	401.16
S3_187450964	3	187450964	0	401.19
S3_187460273	3	187460273	0	401.50
S3_192021444	3	192021444	0	410.40
S3_193756629	3	193756629	0	410.72
S3_192021474	3	192021474	0	411.15
S3_193604422	3	193604422	0	411.93
S3_195700423	3	195700423	0	416.69
S3_196090846	3	196090846	0	421.07
S3_196090908	3	196090908	1	421.15
S3_205364547	3	205364547	1	447.69
S3_205372968	3	205372968	0	447.78
S3_206605750	3	206605750	0	450.34
S3_206690202	3	206690202	0	453.68
S3_206690186	3	206690186	0	454.30
S3_206952500	3	206952500	0	455.16
S3_206943368	3	206943368	0	455.35
S3_207170866	3	207170866	0	456.06

Table A.2 continued.

S3_207147899	3	207147899	0	456.16
S3_206836424	3	206836424	0	457.13
S3_207766005	3	207766005	0	458.60
S3_207988802	3	207988802	0	458.77
S3_208035004	3	208035004	0	459.55
S3_208046297	3	208046297	0	459.82
S3_208048110	3	208048110	0	459.86
S3_208046277	3	208046277	0	459.99
S3_208048092	3	208048092	0	460.11
S3_208048045	3	208048045	0	460.23
S3_208063573	3	208063573	0	460.91
S3_208331031	3	208331031	0	461.61
S3_208901937	3	208901937	0	463.05
S3_208629545	3	208629545	0	463.83
S3_209022752	3	209022752	0	465.67
S3_208941816	3	208941816	0	466.10
S3_208968336	3	208968336	0	466.44
S3_208968342	3	208968342	0	466.61
S3_208941844	3	208941844	0	466.69
S3_209067939	3	209067939	0	467.66
S3_209846954	3	209846954	0	468.73
S3_209846906	3	209846906	0	469.01
S3_209846907	3	209846907	0	469.15
S3_210141735	3	210141735	0	469.32
S3_210065213	3	210065213	0	469.40
S3_210265825	3	210265825	0	469.58
S3_210537583	3	210537583	0	470.10
S3_210558730	3	210558730	0	470.27
S3_210530303	3	210530303	0	470.52
S3_210235070	3	210235070	0	472.26
S3_210235330	3	210235330	0	473.51
S3_210265629	3	210265629	0	473.59
S3_209656089	3	209656089	0	473.59
S3_209724592	3	209724592	0	473.76
S3_210526491	3	210526491	0	473.76
S3_210527426	3	210527426	0	473.76
S3_210526514	3	210526514	0	473.76
S3_210749273	3	210749273	0	475.40
S3_210749284	3	210749284	0	475.57
S3_210749283	3	210749283	0	475.65
S3_210747237	3	210747237	0	475.65
S3_213558460	3	213558460	0	479.39
S3_213548536	3	213548536	0	479.87
S3_213594164	3	213594164	0	480.24
S3_213559752	3	213559752	0	480.52
S3_214372809	3	214372809	0	482.24
S3_214372785	3	214372785	0	482.75
S3_214372782	3	214372782	0	482.75
S3_214372778	3	214372778	0	482.84
S3_214374036	3	214374036	0	483.02
S3_214364687	3	214364687	0	483.29
S3_214902340	3	214902340	0	487.15
S3_214902322	3	214902322	0	487.15
S3_214902774	3	214902774	0	487.32
S3_215475112	3	215475112	0	488.66
S3_215475103	3	215475103	0	489.09
S3_215471266	3	215471266	0	489.52
S3_215663602	3	215663602	0	490.58
S3_215758065	3	215758065	0	490.75
S3_215758011	3	215758011	0	490.75
S3_215786813	3	215786813	0	491.18
S3_215932071	3	215932071	0	491.96
S3_216321612	3	216321612	0	493.21
S3_216257541	3	216257541	0	493.47
S3_216256868	3	216256868	0	493.74
S3_216257614	3	216257614	0	493.81
S3_216257448	3	216257448	0	493.81
S3_216257315	3	216257315	0	494.50
S3_216006137	3	216006137	0	495.56
S3_216472293	3	216472293	0	497.61
S3_216479386	3	216479386	0	498.63
S3_216482957	3	216482957	0	499.11
S3_216988860	3	216988860	0	499.56
S3_216527900	3	216527900	0	500.14
S3_216648247	3	216648247	0	500.26
S3_216528163	3	216528163	0	501.12
S3_216876076	3	216876076	0	501.44
S3_216876325	3	216876325	0	501.53
S3_216841549	3	216841549	0	502.10
S3_216647852	3	216647852	0	502.14
S3_216989160	3	216989160	0	502.35
S3_217237920	3	217237920	0	504.21
S3_217293321	3	217293321	0	505.28

Table A.2 continued.

S3_217462921	3	217462921	0	515.30
S3_217939362	3	217939362	0	524.95
S3_217637698	3	217637698	0	528.10
S3_219373746	3	219373746	0	532.38
S3_219293551	3	219293551	0	532.87
S3_219578008	3	219578008	0	535.24
S3_220704168	3	220704168	0	538.83
S3_220809854	3	220809854	0	539.26
S3_220844515	3	220844515	0	540.53
S3_222426463	3	222426463	0	549.76
S3_221923428	3	221923428	0	550.13
S3_223089043	3	223089043	0	550.92
S3_223309794	3	223309794	0	558.35
S3_221639629	3	221639629	0	572.62
S3_221547197	3	221547197	0	574.82
S3_223090803	3	223090803	0	578.37
S3_222949935	3	222949935	0	579.31
S3_223560357	3	223560357	0	580.29
S3_223996157	3	223996157	0	583.70
S3_225204951	3	225204951	0	591.35
S3_225898255	3	225898255	0	594.75
S3_225898385	3	225898385	0	594.83
S3_226262161	3	226262161	0	595.10
S3_226477643	3	226477643	0	595.53
S3_227190155	3	227190155	0	601.41
S3_228449827	3	228449827	0	606.03
S3_228374771	3	228374771	0	608.51
S3_228374207	3	228374207	0	608.60
S3_228375304	3	228375304	0	608.93
S3_227824614	3	227824614	0	609.30
S3_227824642	3	227824642	0	609.31
S3_228374852	3	228374852	0	609.61
S3_228449587	3	228449587	0	610.04
S3_228551502	3	228551502	0	610.04
S3_229196449	3	229196449	0	613.76
S3_229034931	3	229034931	0	615.14
S3_229867327	3	229867327	0	618.15
S3_230179628	3	230179628	0	619.99
S3_230337672	3	230337672	0	620.87
S3_230252260	3	230252260	0	621.21
S3_230340836	3	230340836	0	621.90
S3_230512903	3	230512903	0	622.72
S3_230991997	3	230991997	0	624.53
S3_230992053	3	230992053	0	624.79
S3_230442601	3	230442601	1	627.58
S4_2409561	4	2409561	1	5.61
S4_2409577	4	2409577	0	6.30
S4_2651122	4	2651122	0	12.66
S4_2831321	4	2831321	0	14.35
S4_2831256	4	2831256	0	14.82
S4_2831316	4	2831316	0	14.91
S4_3746503	4	3746503	0	23.14
S4_3746752	4	3746752	0	23.44
S4_4272122	4	4272122	0	26.66
S4_4272138	4	4272138	0	26.84
S4_4271267	4	4271267	0	26.95
S4_4271229	4	4271229	0	29.65
S4_4271182	4	4271182	0	29.76
S4_4395352	4	4395352	0	30.75
S4_4395534	4	4395534	0	32.35
S4_5245749	4	5245749	1	45.21
S4_5708678	4	5708678	1	46.29
S4_5314307	4	5314307	0	51.58
S4_5314300	4	5314300	0	52.09
Zeinssr1	4	5000000	0	55.82
UMC2150	4	5375803	0	59.79
S4_5959741	4	5959741	0	65.83
S4_8676015	4	8676015	0	72.85
S4_9631857	4	9631857	0	74.03
S4_9732977	4	9732977	0	74.85
S4_9732920	4	9732920	0	74.94
S4_10033504	4	10033504	0	81.63
S4_10901447	4	10901447	0	90.25
S4_11647026	4	11647026	0	93.36
S4_12977118	4	12977118	0	95.51
S4_12782043	4	12782043	0	96.57
Phi021	4	13395375	0	99.47
S4_13350518	4	13350518	0	102.48
S4_13688723	4	13688723	0	102.74
S4_13688505	4	13688505	0	102.74
S4_13688235	4	13688235	0	102.74
S4_13946665	4	13946665	0	104.08
S4_14041207	4	14041207	0	105.24

Table A.2 continued.

S4_14724798	4	14724798	0	112.93
S4_14722372	4	14722372	0	113.10
S4_17331853	4	17331853	0	115.03
S4_17331867	4	17331867	0	115.72
S4_16847475	4	16847475	0	116.51
S4_16847526	4	16847526	0	116.73
S4_16603452	4	16603452	0	117.28
S4_16613072	4	16613072	0	117.40
S4_16604845	4	16604845	0	118.70
S4_16984985	4	16984985	0	119.70
S4_17142438	4	17142438	0	120.04
S4_17142383	4	17142383	0	120.16
S4_17375434	4	17375434	0	120.82
S4_17500704	4	17500704	0	121.51
S4_17829155	4	17829155	0	122.33
S4_17855227	4	17855227	0	122.67
S4_17855158	4	17855158	0	122.67
S4_17742333	4	17742333	0	122.80
S4_17830507	4	17830507	0	122.93
S4_17830498	4	17830498	0	123.06
S4_18581648	4	18581648	0	125.11
S4_18809248	4	18809248	0	125.45
S4_18911790	4	18911790	0	125.45
S4_19945678	4	19945678	0	126.26
S4_20776685	4	20776685	0	128.08
S4_20409883	4	20409883	0	128.16
S4_20406000	4	20406000	0	128.33
S4_20409800	4	20409800	0	128.50
S4_20908503	4	20908503	0	128.91
S4_21320418	4	21320418	0	129.14
S4_21823230	4	21823230	0	130.95
S4_25464678	4	25464678	0	134.91
S4_24905927	4	24905927	0	135.26
S4_24113584	4	24113584	0	135.59
S4_25071797	4	25071797	0	135.69
S4_23529223	4	23529223	0	136.20
S4_23166593	4	23166593	0	137.08
S4_34654468	4	34654468	0	141.29
S4_34873510	4	34873510	0	141.39
S4_34803513	4	34803513	0	141.65
S4_34055291	4	34055291	0	142.54
S4_34666736	4	34666736	0	143.15
S4_28980793	4	28980793	0	144.88
S4_28115569	4	28115569	0	145.67
S4_27750215	4	27750215	0	145.92
S4_27455041	4	27455041	0	146.00
S4_27568048	4	27568048	0	146.00
S4_28983780	4	28983780	0	146.79
S4_29128089	4	29128089	0	147.05
S4_29126182	4	29126182	0	147.05
S4_30652638	4	30652638	0	147.74
S4_30199166	4	30199166	0	148.30
S4_31123080	4	31123080	0	149.14
S4_31674592	4	31674592	0	150.20
S4_33116203	4	33116203	0	151.36
S4_32811665	4	32811665	0	151.36
S4_32813138	4	32813138	0	151.36
S4_33116267	4	33116267	0	151.45
S4_34932345	4	34932345	0	152.60
S4_35005077	4	35005077	0	152.60
S4_35005091	4	35005091	0	152.69
S4_35005165	4	35005165	0	152.85
S4_35102085	4	35102085	0	153.55
S4_35102094	4	35102094	0	153.76
S4_36314067	4	36314067	0	154.52
S4_34875181	4	34875181	0	155.47
S4_34804248	4	34804248	0	155.64
S4_34873508	4	34873508	0	155.64
S4_34875439	4	34875439	0	155.75
S4_36217059	4	36217059	0	156.33
S4_36041177	4	36041177	0	156.41
S4_36041162	4	36041162	0	156.50
S4_36311329	4	36311329	0	156.50
S4_36409404	4	36409404	0	156.58
S4_35920536	4	35920536	0	156.83
S4_35102175	4	35102175	0	156.92
S4_35985028	4	35985028	0	157.00
S4_35102160	4	35102160	0	157.09
S4_35102200	4	35102200	0	157.20
S4_73366233	4	73366233	0	159.21
S4_75694818	4	75694818	0	160.16
S4_75694927	4	75694927	0	160.26
S4_76277190	4	76277190	0	160.26

Table A.2 continued.

S4_75695092	4	75695092	0	160.43
S4_74269198	4	74269198	0	160.44
S4_73366918	4	73366918	0	160.86
S4_74269125	4	74269125	0	161.11
S4_73773182	4	73773182	0	161.20
S4_73422080	4	73422080	0	161.20
S4_73367184	4	73367184	0	161.20
S4_73773142	4	73773142	0	161.20
S4_78545065	4	78545065	0	161.54
S4_78545053	4	78545053	0	161.71
S4_81251401	4	81251401	0	162.05
S4_79589402	4	79589402	0	162.38
S4_77454220	4	77454220	0	162.73
S4_79106228	4	79106228	0	163.16
S4_79106236	4	79106236	0	163.24
S4_79449620	4	79449620	0	163.25
S4_81592460	4	81592460	0	164.03
S4_82014336	4	82014336	0	164.74
S4_82359533	4	82359533	0	165.15
S4_82359387	4	82359387	0	165.26
S4_85296701	4	85296701	0	166.11
S4_86271372	4	86271372	0	166.21
S4_93244318	4	93244318	0	166.44
S4_113417283	4	113417283	0	167.12
S4_114276437	4	114276437	0	167.47
S4_120988951	4	120988951	0	168.04
S4_120887175	4	120887175	0	168.21
S4_120596986	4	120596986	0	168.31
S4_120988860	4	120988860	0	168.72
S4_124280815	4	124280815	0	172.20
S4_124279854	4	124279854	0	172.20
S4_124279802	4	124279802	0	172.28
S4_124279801	4	124279801	0	172.45
S4_128990791	4	128990791	0	173.09
S4_127831597	4	127831597	0	173.34
S4_127011982	4	127011982	0	173.68
S4_127208538	4	127208538	0	174.28
S4_126473756	4	126473756	0	174.82
S4_121563375	4	121563375	0	175.14
S4_126342572	4	126342572	0	175.14
S4_126342571	4	126342571	0	175.14
S4_126342574	4	126342574	0	175.22
S4_126474032	4	126474032	0	175.48
S4_126342567	4	126342567	0	175.65
S4_126342566	4	126342566	0	175.82
S4_126342569	4	126342569	0	175.90
S4_126473591	4	126473591	0	176.15
S4_126341881	4	126341881	0	176.24
S4_127208585	4	127208585	0	176.58
S4_127189841	4	127189841	0	176.67
S4_131765746	4	131765746	0	177.63
S4_133253103	4	133253103	0	178.98
S4_134623126	4	134623126	0	179.10
S4_133253504	4	133253504	0	179.22
S4_131770242	4	131770242	0	179.34
S4_130031697	4	130031697	0	180.12
S4_128603647	4	128603647	0	180.37
S4_128603645	4	128603645	0	180.46
S4_130460021	4	130460021	0	180.72
S4_130433562	4	130433562	0	180.80
S4_130433686	4	130433686	0	180.94
S4_130433433	4	130433433	0	181.27
S4_130431471	4	130431471	0	181.44
S4_127925630	4	127925630	0	183.28
S4_141413145	4	141413145	0	184.06
S4_141477171	4	141477171	0	184.32
S4_136068022	4	136068022	0	184.32
S4_136068058	4	136068058	0	184.40
S4_136068046	4	136068046	0	184.49
S4_136068030	4	136068030	0	184.49
S4_141279771	4	141279771	0	184.57
S4_141096491	4	141096491	0	184.57
S4_141279715	4	141279715	0	184.57
S4_141096388	4	141096388	0	184.74
S4_133820555	4	133820555	0	185.26
S4_142386714	4	142386714	0	186.89
S4_142384853	4	142384853	0	187.95
S4_143082419	4	143082419	0	188.65
S4_143082453	4	143082453	0	188.81
S4_143379653	4	143379653	0	189.05
S4_143379620	4	143379620	0	189.07
S4_143082499	4	143082499	0	189.18
S4_143082905	4	143082905	0	189.41

Table A.2 continued.

S4_143082861	4	143082861	0	189.56
S4_143082455	4	143082455	0	189.72
S4_143379638	4	143379638	0	189.86
S4_143804660	4	143804660	0	190.41
S4_143804647	4	143804647	0	191.10
S4_143804657	4	143804657	1	191.19
S4_144043686	4	144043686	1	191.74
S4_144727138	4	144727138	0	194.01
S4_144532043	4	144532043	0	194.10
S4_144732519	4	144732519	0	194.43
S4_144283698	4	144283698	0	195.06
S4_144281174	4	144281174	0	195.45
S4_146977432	4	146977432	0	195.45
S4_150813779	4	150813779	0	206.84
S4_149745262	4	149745262	0	208.14
S4_149745238	4	149745238	0	208.15
S4_149745215	4	149745215	1	208.40
S4_156023565	4	156023565	1	223.01
S4_156516774	4	156516774	0	225.45
S4_156513274	4	156513274	0	226.06
S4_156518337	4	156518337	0	226.66
S4_157113682	4	157113682	0	227.61
S4_157112007	4	157112007	0	227.71
S4_157107764	4	157107764	0	228.17
S4_157119223	4	157119223	0	228.57
S4_157119184	4	157119184	0	228.72
S4_157119182	4	157119182	0	229.02
S4_157119225	4	157119225	0	229.19
S4_158146568	4	158146568	0	230.93
S4_161500101	4	161500101	0	235.41
S4_162762547	4	162762547	0	236.15
S4_162907836	4	162907836	0	236.32
S4_162762794	4	162762794	0	236.86
S4_162762885	4	162762885	0	237.24
S4_162762882	4	162762882	0	237.63
S4_163201708	4	163201708	0	239.27
S4_163448154	4	163448154	0	239.97
S4_163397115	4	163397115	0	240.41
S4_163779660	4	163779660	0	241.28
S4_164174155	4	164174155	0	242.42
S4_165818277	4	165818277	0	243.80
S4_166913200	4	166913200	0	245.83
S4_166913229	4	166913229	0	245.92
S4_166913202	4	166913202	0	246.01
S4_166913199	4	166913199	0	246.25
S4_166913198	4	166913198	0	246.42
S4_166913895	4	166913895	0	246.68
S4_166913910	4	166913910	0	246.76
S4_166911372	4	166911372	0	247.37
S4_166907913	4	166907913	0	247.82
S4_165993841	4	165993841	0	250.38
S4_165841691	4	165841691	0	250.64
S4_164263827	4	164263827	0	251.87
S4_164262054	4	164262054	0	252.17
S4_159570395	4	159570395	0	261.77
S4_159871397	4	159871397	0	262.20
S4_159176618	4	159176618	0	262.37
S4_159667125	4	159667125	0	262.70
S4_159725793	4	159725793	0	262.80
S4_158705481	4	158705481	0	263.05
S4_159830109	4	159830109	0	263.31
S4_159724527	4	159724527	0	263.48
S4_158862960	4	158862960	0	263.65
S4_158705473	4	158705473	0	263.73
S4_159830106	4	159830106	0	263.81
S4_159570387	4	159570387	0	263.81
S4_159978445	4	159978445	0	271.15
S4_156396121	4	156396121	1	271.15
S4_172996746	4	172996746	1	309.77
S4_172996351	4	172996351	0	310.13
S4_173719478	4	173719478	0	312.49
S4_175278618	4	175278618	0	314.13
S4_175278636	4	175278636	0	314.39
S4_175278543	4	175278543	0	314.47
S4_175277962	4	175277962	0	314.73
S4_175277953	4	175277953	0	314.90
S4_177122337	4	177122337	0	317.91
S4_177328630	4	177328630	0	319.07
S4_177323406	4	177323406	0	319.76
S4_177150234	4	177150234	0	320.10
S4_179710916	4	179710916	0	326.45
S4_179617558	4	179617558	0	326.88
S4_179617572	4	179617572	0	326.99

Table A.2 continued.

S4_179615385	4	179615385	0	327.56
S4_179629726	4	179629726	0	327.99
S4_179617550	4	179617550	0	328.87
S4_179661800	4	179661800	0	329.71
S4_179661316	4	179661316	0	329.91
S4_180446392	4	180446392	0	331.01
S4_180238607	4	180238607	0	331.50
S4_180433531	4	180433531	0	332.38
S4_180433582	4	180433582	0	332.46
S4_180433446	4	180433446	0	332.80
S4_180433500	4	180433500	0	333.06
S4_180198556	4	180198556	0	333.44
S4_179804795	4	179804795	1	336.51
S4_184396335	4	184396335	1	347.19
S4_184478971	4	184478971	0	347.46
S4_184396426	4	184396426	1	347.63
S4_185690252	4	185690252	1	350.64
S4_185695207	4	185695207	0	350.91
S4_185714230	4	185714230	1	351.18
S4_198430268	4	198430268	1	380.76
S4_198135495	4	198135495	0	381.31
S4_203098582	4	203098582	0	384.77
S4_197072950	4	197072950	0	393.51
S4_197072985	4	197072985	0	394.03
S4_197082206	4	197082206	0	394.45
S4_197524946	4	197524946	0	394.72
S4_197853756	4	197853756	0	395.64
S4_198079421	4	198079421	0	396.46
S4_198079415	4	198079415	0	397.16
S4_198079418	4	198079418	0	397.38
S4_198082028	4	198082028	0	397.57
S4_200032049	4	200032049	0	401.68
S4_200034074	4	200034074	0	401.86
S4_199711857	4	199711857	0	402.03
S4_199598187	4	199598187	0	402.55
S4_199785143	4	199785143	0	402.89
S4_199797535	4	199797535	0	402.97
S4_200031945	4	200031945	0	403.14
S4_200189714	4	200189714	0	403.57
S4_200185395	4	200185395	0	404.18
S4_200837734	4	200837734	0	405.05
S4_200842659	4	200842659	0	405.05
S4_201751614	4	201751614	0	405.48
S4_201751608	4	201751608	0	405.56
S4_200185422	4	200185422	0	405.84
S4_191579817	4	191579817	0	412.02
S4_189815210	4	189815210	0	418.53
S4_189815133	4	189815133	0	418.62
S4_189815129	4	189815129	0	418.85
S4_189815152	4	189815152	0	419.05
S4_189815206	4	189815206	0	419.39
S4_189815491	4	189815491	0	419.73
S4_189904490	4	189904490	0	419.99
S4_190262923	4	190262923	0	420.94
S4_189814989	4	189814989	0	421.54
S4_189814971	4	189814971	0	421.81
S4_189814998	4	189814998	0	422.15
S4_189814965	4	189814965	0	422.15
S4_189176427	4	189176427	0	423.08
S4_188876211	4	188876211	0	424.31
S4_188876237	4	188876237	0	424.82
S4_188876233	4	188876233	0	425.54
S4_189036252	4	189036252	0	425.88
S4_189157835	4	189157835	0	425.88
S4_188877625	4	188877625	0	426.05
S4_189017163	4	189017163	0	426.48
S4_188412264	4	188412264	0	426.91
S4_188412166	4	188412166	0	426.98
S4_188487326	4	188487326	0	427.06
S4_188704932	4	188704932	0	427.15
S4_189294831	4	189294831	0	427.85
S4_189294989	4	189294989	0	428.37
S4_188208937	4	188208937	0	430.74
S4_188300113	4	188300113	0	432.19
S4_186503159	4	186503159	0	436.16
S4_186482025	4	186482025	0	436.34
S4_186352314	4	186352314	0	437.03
S4_186503109	4	186503109	0	437.45
S4_186481111	4	186481111	0	437.45
S4_186503118	4	186503118	0	437.45
S4_186942512	4	186942512	0	438.89
S4_186942452	4	186942452	0	438.89
S4_187417472	4	187417472	0	440.34

Table A.2 continued.

S4_187875468	4	187875468	0	441.59
S4_187875467	4	187875467	0	441.76
S4_187604347	4	187604347	0	444.18
S4_187417835	4	187417835	0	445.01
S4_187268942	4	187268942	0	445.46
S4_186644779	4	186644779	0	446.42
S4_187296983	4	187296983	0	447.29
S4_187277944	4	187277944	0	447.55
S4_187290470	4	187290470	0	447.64
S4_187301712	4	187301712	0	447.64
S4_187296722	4	187296722	0	447.64
S4_187290467	4	187290467	0	447.72
S4_191720351	4	191720351	0	453.31
S4_191720346	4	191720346	0	453.40
S4_194316824	4	194316824	0	456.41
S4_193350931	4	193350931	0	457.02
S4_193350864	4	193350864	0	457.18
S4_196984902	4	196984902	0	458.47
S4_196987543	4	196987543	0	458.62
S4_196987540	4	196987540	0	458.73
S4_195798203	4	195798203	0	459.72
S4_195004315	4	195004315	0	461.12
S4_196824037	4	196824037	0	462.24
S4_193344522	4	193344522	0	463.30
S4_193226235	4	193226235	0	463.39
S4_193147210	4	193147210	0	463.48
S4_195004401	4	195004401	0	463.98
S4_192217274	4	192217274	0	465.09
S4_197563807	4	197563807	0	468.00
S4_197370067	4	197370067	0	468.69
S4_197388600	4	197388600	0	468.77
S4_189295619	4	189295619	0	471.61
S4_204382730	4	204382730	0	476.68
S4_206077408	4	206077408	0	477.95
S4_206081233	4	206081233	0	478.20
S4_210559426	4	210559426	0	478.48
S4_212596661	4	212596661	0	479.26
S4_213998594	4	213998594	0	479.69
S4_214378730	4	214378730	0	479.86
S4_213998749	4	213998749	0	480.11
S4_214034873	4	214034873	0	480.14
S4_213777313	4	213777313	0	480.92
S4_214507055	4	214507055	0	481.26
S4_214507097	4	214507097	0	482.19
S4_215103643	4	215103643	0	482.73
S4_215103639	4	215103639	0	483.07
S4_215038861	4	215038861	0	483.07
S4_214912863	4	214912863	0	483.24
S4_217026123	4	217026123	0	484.21
S4_216321651	4	216321651	0	485.13
S4_204208972	4	204208972	0	490.26
S4_205977721	4	205977721	0	491.87
S4_223519868	4	223519868	0	495.95
S4_227728349	4	227728349	0	499.57
S4_227889467	4	227889467	0	499.66
S4_228554104	4	228554104	0	500.79
S4_228554454	4	228554454	0	500.87
S4_228623100	4	228623100	0	501.12
S4_228623026	4	228623026	0	501.20
S4_226621108	4	226621108	0	504.00
S4_226457943	4	226457943	0	504.61
S4_226457942	4	226457942	0	504.78
S4_226305515	4	226305515	0	505.13
S4_226305542	4	226305542	1	505.22
S4_233203593	4	233203593	1	521.27
S4_233203594	4	233203594	0	525.71
S4_233089784	4	233089784	0	532.54
S4_233070870	4	233070870	0	535.67
S4_233757495	4	233757495	0	539.77
S4_233635129	4	233635129	0	539.94
S4_233629566	4	233629566	0	540.10
S4_233757271	4	233757271	0	540.27
S4_233210730	4	233210730	1	548.38
S4_236479062	4	236479062	1	555.98
S4_237041279	4	237041279	0	568.34
S4_237041321	4	237041321	0	568.68
S4_236951909	4	236951909	0	568.85
S4_237312082	4	237312082	0	571.32
S4_237319593	4	237319593	0	571.57
S4_237317948	4	237317948	0	571.91
S4_237317946	4	237317946	0	572.00
S4_237146991	4	237146991	0	573.25
S4_237146974	4	237146974	0	574.04

Table A.2 continued.

S4_237146983	4	237146983	0	574.30
S4_237391758	4	237391758	0	577.42
S4_237390617	4	237390617	0	577.76
S4_237390630	4	237390630	0	577.84
S4_237499150	4	237499150	0	579.78
S4_237499147	4	237499147	0	580.12
S4_237499145	4	237499145	0	580.38
S4_237503060	4	237503060	0	582.02
S4_237503063	4	237503063	0	582.19
S4_236169774	4	236169774	0	587.48
S4_236371128	4	236371128	0	587.74
S4_236371137	4	236371137	0	587.82
S4_236184651	4	236184651	0	588.37
S4_236091115	4	236091115	0	589.12
S4_236091105	4	236091105	0	589.51
S4_235728621	4	235728621	0	590.57
S4_235719658	4	235719658	0	591.64
S4_235719666	4	235719666	0	592.07
S4_235210936	4	235210936	0	593.84
S4_235210944	4	235210944	0	594.01
S4_235202974	4	235202974	0	594.89
S4_235202921	4	235202921	0	595.06
S4_234873496	4	234873496	0	596.13
S4_234447740	4	234447740	0	599.61
S4_234420709	4	234420709	0	601.56
S4_234420710	4	234420710	0	601.74
S4_234419646	4	234419646	0	602.13
S4_235380629	4	235380629	0	607.26
S4_235299121	4	235299121	0	607.77
S4_235452943	4	235452943	0	608.12
S4_235683793	4	235683793	0	609.75
S4_235826564	4	235826564	0	611.19
S4_237517605	4	237517605	0	621.50
S4_237517546	4	237517546	0	621.59
S4_237521486	4	237521486	0	621.85
S4_237611655	4	237611655	0	631.68
S4_237687561	4	237687561	0	632.11
S4_237773416	4	237773416	0	634.26
S4_237887622	4	237887622	0	634.69
S4_237887634	4	237887634	0	636.33
S4_237898483	4	237898483	0	636.33
S4_237898681	4	237898681	0	636.42
S4_238168312	4	238168312	0	640.77
S4_238168361	4	238168361	0	640.85
S4_238168314	4	238168314	0	640.85
S4_238121384	4	238121384	0	648.72
S4_238762461	4	238762461	0	660.40
S4_239001389	4	239001389	0	662.48
S4_239010058	4	239010058	0	663.14
S4_239935988	4	239935988	0	670.22
S4_239703676	4	239703676	0	671.66
S4_239704653	4	239704653	0	671.66
S4_239705058	4	239705058	0	672.36
S4_239703345	4	239703345	0	672.88
S4_239703247	4	239703247	0	673.05
S4_239703749	4	239703749	0	673.14
S4_239793038	4	239793038	0	674.52
S4_239797119	4	239797119	0	674.74
S4_240226594	4	240226594	0	679.27
S4_241030685	4	241030685	1	682.51
S5_1949966	5	1949966	1	5.53
S5_2139966	5	2139966	0	7.09
S5_2120221	5	2120221	0	8.12
S5_2120268	5	2120268	0	8.12
S5_2120710	5	2120710	0	8.55
S5_2250736	5	2250736	1	14.16
S5_7575712	5	7575712	1	29.25
S5_8175868	5	8175868	0	32.90
S5_8331697	5	8331697	0	33.87
S5_9376590	5	9376590	0	36.12
S5_8541536	5	8541536	0	38.17
S5_8426641	5	8426641	0	38.69
S5_9622026	5	9622026	0	41.27
S5_10106056	5	10106056	0	45.36
S5_10091304	5	10091304	0	47.84
S5_10100300	5	10100300	0	49.10
S5_10412391	5	10412391	0	51.70
S5_10273708	5	10273708	0	52.57
S5_10418157	5	10418157	0	53.76
S5_10901534	5	10901534	0	53.85
S5_10705494	5	10705494	0	53.99
S5_11086295	5	11086295	0	54.28
S5_11594195	5	11594195	0	55.06

Table A.2 continued.

S8_165340850	8	165340850	0	55.84
S5_11363645	5	11363645	0	58.76
S5_10898741	5	10898741	0	60.12
S5_10898737	5	10898737	0	60.12
S5_10542684	5	10542684	0	62.37
S5_10541117	5	10541117	0	62.88
S5_10705595	5	10705595	0	62.96
S5_10417899	5	10417899	0	64.62
S5_12231111	5	12231111	0	72.15
S5_12230939	5	12230939	0	72.61
S5_12385604	5	12385604	0	73.26
S5_12291521	5	12291521	0	73.67
S5_12270414	5	12270414	0	74.03
S5_14057904	5	14057904	0	77.16
S5_13970445	5	13970445	0	77.86
S5_13970424	5	13970424	0	77.96
S5_13738465	5	13738465	0	77.96
S5_13807499	5	13807499	0	78.13
S5_13970359	5	13970359	0	78.22
S5_13803054	5	13803054	0	78.38
S5_13970508	5	13970508	0	78.55
S5_13947708	5	13947708	0	78.89
S5_13721708	5	13721708	0	79.50
S5_14060182	5	14060182	0	80.19
S5_14060229	5	14060229	0	81.16
S5_14138435	5	14138435	0	81.85
S5_14138394	5	14138394	0	82.12
S5_18276883	5	18276883	0	89.46
S5_17229164	5	17229164	0	89.88
S5_18152784	5	18152784	0	90.14
S5_18152898	5	18152898	0	90.14
S5_18156789	5	18156789	0	90.39
S5_18275928	5	18275928	0	90.48
S5_18152887	5	18152887	0	91.11
S5_18572178	5	18572178	0	96.25
S5_15462072	5	15462072	0	103.73
S5_15448445	5	15448445	0	104.21
S5_15436035	5	15436035	0	104.48
S5_14609277	5	14609277	0	111.18
S5_14588659	5	14588659	0	112.94
S7_25900352	7	25900352	0	114.54
S5_14925764	5	14925764	1	121.05
S5_23268761	5	23268761	1	144.70
S5_23268742	5	23268742	0	144.87
S5_23573801	5	23573801	0	145.58
S5_23161093	5	23161093	0	145.94
S5_23267249	5	23267249	0	146.14
S5_23267306	5	23267306	0	146.45
S5_23874281	5	23874281	0	147.40
S5_23838550	5	23838550	0	147.58
S5_23874180	5	23874180	0	147.66
S5_24714711	5	24714711	0	149.11
S5_24969786	5	24969786	0	149.37
S5_25400156	5	25400156	0	149.88
S5_25272215	5	25272215	1	150.49
S5_29707811	5	29707811	1	163.07
S5_29707410	5	29707410	0	163.43
S5_29991183	5	29991183	0	163.85
S5_29918567	5	29918567	0	167.00
S5_27945784	5	27945784	0	168.42
S5_27099745	5	27099745	0	171.15
S5_26100208	5	26100208	0	172.32
S5_26660025	5	26660025	0	172.68
S5_27861969	5	27861969	0	175.36
S5_44304115	5	44304115	0	182.98
S5_43925429	5	43925429	0	183.06
S5_44304132	5	44304132	0	183.25
S5_44404151	5	44404151	0	183.43
S5_51667851	5	51667851	0	186.66
S5_39881801	5	39881801	0	195.36
S5_39883773	5	39883773	0	197.75
S5_39883706	5	39883706	0	198.04
S5_37256448	5	37256448	0	198.97
S5_37845443	5	37845443	0	198.97
S5_37706692	5	37706692	0	199.05
S5_38263377	5	38263377	0	199.14
S5_37719931	5	37719931	0	199.14
S5_38202517	5	38202517	0	199.31
S5_38261649	5	38261649	0	199.56
S5_32874548	5	32874548	0	200.68
S5_32848386	5	32848386	0	200.97
S5_32798131	5	32798131	0	201.22
S5_32095057	5	32095057	0	201.74

Table A.2 continued.

S5_32090529	5	32090529	0	202.43
S5_32227047	5	32227047	0	203.22
S5_40835307	5	40835307	0	204.00
S5_37256357	5	37256357	0	204.62
S5_37256242	5	37256242	0	204.75
S5_37256237	5	37256237	0	205.30
S5_70776941	5	70776941	0	211.56
S5_70869380	5	70869380	0	211.88
S5_70870679	5	70870679	0	212.31
S5_70774939	5	70774939	0	212.31
S5_70870353	5	70870353	0	212.40
S5_70870604	5	70870604	0	212.48
S5_69452832	5	69452832	0	212.92
S5_70063644	5	70063644	0	213.17
S5_70063724	5	70063724	0	213.26
S5_70773399	5	70773399	0	214.04
S5_80804488	5	80804488	0	219.42
S5_80807971	5	80807971	0	219.89
S5_84104174	5	84104174	0	221.43
S5_84104987	5	84104987	0	221.51
S5_84104404	5	84104404	0	221.51
S5_84063998	5	84063998	0	221.68
S5_133505829	5	133505829	0	228.47
S5_133776621	5	133776621	0	228.65
S5_137393011	5	137393011	0	229.63
S5_75935777	5	75935777	0	238.14
S5_82423678	5	82423678	0	242.90
S5_82234158	5	82234158	0	247.28
S5_81863240	5	81863240	0	247.28
S5_82069433	5	82069433	0	247.36
S5_84841805	5	84841805	0	247.96
S5_82069429	5	82069429	0	249.02
S5_80969998	5	80969998	0	250.30
S5_80970003	5	80970003	0	250.62
S5_80971601	5	80971601	0	250.79
S5_145496748	5	145496748	0	260.02
S5_89156735	5	89156735	0	267.91
S5_89156774	5	89156774	1	268.08
S5_89450176	5	89450176	1	268.91
S5_89450175	5	89450175	1	269.09
S5_156351470	5	156351470	1	458.73
S5_156754020	5	156754020	0	459.08
S5_157444481	5	157444481	0	460.52
S5_157463064	5	157463064	0	460.61
S5_157496819	5	157496819	0	460.78
S5_157446087	5	157446087	0	461.10
S5_157446027	5	157446027	0	461.10
S5_157448043	5	157448043	0	461.26
S5_157444503	5	157444503	0	461.48
S5_157496744	5	157496744	0	461.94
S5_157496745	5	157496745	0	462.03
S5_157456160	5	157456160	0	462.63
S5_158821395	5	158821395	0	470.02
S5_163890137	5	163890137	0	475.07
S5_164861453	5	164861453	0	477.76
S5_164989812	5	164989812	0	479.19
S5_165071094	5	165071094	0	479.49
S5_166332396	5	166332396	0	482.12
S5_166218251	5	166218251	0	482.91
S5_166218205	5	166218205	0	483.07
S5_166515146	5	166515146	0	483.78
S5_166515127	5	166515127	0	483.86
S5_166473857	5	166473857	0	484.03
S5_166855823	5	166855823	0	484.72
S5_166969946	5	166969946	0	484.72
S5_166969956	5	166969956	0	484.89
S5_166969958	5	166969958	0	484.98
S5_166934277	5	166934277	0	485.23
S5_166969926	5	166969926	0	485.23
S5_167276800	5	167276800	0	486.05
S5_167276742	5	167276742	0	486.18
S5_167068439	5	167068439	0	486.75
S5_167068437	5	167068437	0	486.91
S5_167068335	5	167068335	0	487.08
S5_167276740	5	167276740	0	487.08
S5_167276704	5	167276704	0	487.68
S5_167364201	5	167364201	0	488.64
S5_167585729	5	167585729	0	490.47
S5_168671641	5	168671641	0	492.60
S5_168671638	5	168671638	0	492.80
S5_168667286	5	168667286	0	494.17
S5_167784588	5	167784588	0	494.93
S5_168446836	5	168446836	0	495.54

Table A.2 continued.

S5_168447594	5	168447594	0	496.05
S5_168673209	5	168673209	0	496.31
S5_169814418	5	169814418	0	499.32
S5_169667550	5	169667550	0	500.10
S5_169680561	5	169680561	0	500.19
S5_168893869	5	168893869	0	500.62
S5_168907825	5	168907825	0	500.79
S5_169224663	5	169224663	0	500.79
S5_170826263	5	170826263	0	506.23
S5_173810254	5	173810254	0	514.33
S5_175434204	5	175434204	0	524.06
S5_171706714	5	171706714	0	531.92
S5_171706638	5	171706638	0	532.27
S5_171704988	5	171704988	0	532.45
S5_178244055	5	178244055	0	542.98
S5_178323618	5	178323618	0	544.31
S5_178323676	5	178323676	0	544.39
S5_177843905	5	177843905	0	547.07
S5_176990962	5	176990962	0	551.77
S5_178828748	5	178828748	0	556.08
S5_178828178	5	178828178	0	557.33
S5_178828433	5	178828433	0	557.41
S5_180279724	5	180279724	0	561.29
S5_180903410	5	180903410	0	562.40
S5_181587106	5	181587106	0	563.12
S5_182759876	5	182759876	0	566.02
S5_182760412	5	182760412	0	566.19
S5_182576523	5	182576523	0	566.36
S5_182450672	5	182450672	0	566.45
S5_182584005	5	182584005	0	566.71
S5_182584625	5	182584625	0	566.97
S5_182542047	5	182542047	0	567.14
S5_182051184	5	182051184	0	568.34
S5_182090072	5	182090072	0	568.50
S5_182247093	5	182247093	0	568.67
S5_182137899	5	182137899	0	569.01
S5_182193360	5	182193360	0	569.10
S5_182193358	5	182193358	0	569.18
S5_182248008	5	182248008	0	569.96
S5_183144990	5	183144990	0	571.55
S5_183144997	5	183144997	0	572.81
S5_183618676	5	183618676	0	576.72
S5_185574270	5	185574270	0	583.54
S5_184352687	5	184352687	0	587.10
S5_184394764	5	184394764	0	587.72
S5_184494852	5	184494852	0	588.23
S5_185216317	5	185216317	0	588.84
S5_185500471	5	185500471	0	589.57
S5_186117232	5	186117232	0	590.79
S5_186945308	5	186945308	0	592.13
S5_186945127	5	186945127	0	592.21
S5_195185895	5	195185895	0	592.54
S5_195132860	5	195132860	0	592.64
S5_186938361	5	186938361	0	592.89
S5_186945253	5	186945253	0	592.98
S5_186945353	5	186945353	0	592.98
S5_186495460	5	186495460	0	597.35
S5_186504758	5	186504758	0	598.11
S5_186678625	5	186678625	0	599.69
S4_143803477	4	143803477	0	601.83
S5_187017672	5	187017672	0	602.18
S5_187809098	5	187809098	0	602.54
S5_187085022	5	187085022	0	603.33
S5_188200221	5	188200221	0	605.19
S5_188826825	5	188826825	0	609.54
S5_188825424	5	188825424	0	609.98
S5_188668106	5	188668106	0	610.76
S5_188667256	5	188667256	0	610.87
S5_188667398	5	188667398	0	611.03
S5_189198065	5	189198065	0	611.37
S5_193652476	5	193652476	0	614.59
S5_192222140	5	192222140	0	614.68
S5_193122385	5	193122385	0	615.10
S5_191588487	5	191588487	0	623.33
S5_204525048	5	204525048	0	632.98
S5_204812896	5	204812896	0	641.86
S5_204324106	5	204324106	0	648.22
S5_204207566	5	204207566	0	650.31
S5_200292465	5	200292465	0	663.10
S5_200688715	5	200688715	0	671.33
S5_200688862	5	200688862	0	671.59
S5_200688772	5	200688772	0	671.59
S5_200771156	5	200771156	0	672.10

Table A.2 continued.

S5_200778777	5	200778777	0	672.10
S5_200907642	5	200907642	0	680.71
S5_201470428	5	201470428	0	686.08
S5_202291117	5	202291117	0	686.97
S5_202291114	5	202291114	0	687.24
S5_202291112	5	202291112	1	688.05
S5_203716647	5	203716647	1	692.09
S5_204065025	5	204065025	0	696.77
S5_203043989	5	203043989	0	704.45
S5_203037180	5	203037180	0	704.62
S5_203402671	5	203402671	0	705.04
S5_203547859	5	203547859	0	705.31
S5_203547853	5	203547853	0	705.50
S5_205534548	5	205534548	0	711.97
S5_205388990	5	205388990	0	714.65
S5_204813136	5	204813136	0	723.30
S5_204903819	5	204903819	0	735.57
S5_204866582	5	204866582	0	736.66
S5_205564979	5	205564979	1	743.94
S5_207127752	5	207127752	1	748.37
S5_207137213	5	207137213	0	748.93
S5_207121462	5	207121462	0	749.74
S5_207120570	5	207120570	0	750.93
S5_206742818	5	206742818	0	758.88
S5_208429451	5	208429451	0	767.51
S5_209896867	5	209896867	0	773.25
S5_209909276	5	209909276	0	773.25
S5_209896796	5	209896796	0	773.83
S5_210058754	5	210058754	1	783.76
S5_211538208	5	211538208	1	787.95
S5_211179420	5	211179420	0	790.65
S5_211179376	5	211179376	0	790.90
S5_211179419	5	211179419	0	790.99
S5_210631469	5	210631469	0	793.03
S5_210474170	5	210474170	0	793.21
S5_210739960	5	210739960	0	795.11
S5_210739984	5	210739984	0	795.18
S5_210741041	5	210741041	0	797.58
S5_210881188	5	210881188	0	798.87
S5_210879484	5	210879484	0	798.96
S5_210643579	5	210643579	0	800.02
S5_210869357	5	210869357	0	800.81
S5_210869358	5	210869358	0	801.16
S5_210881178	5	210881178	0	801.25
S5_210869404	5	210869404	0	801.83
S5_210870190	5	210870190	0	801.91
S5_210885567	5	210885567	0	801.91
S5_210890517	5	210890517	0	802.34
S5_210890488	5	210890488	0	802.62
S5_211283572	5	211283572	0	804.33
S5_211443649	5	211443649	0	804.85
S5_211446066	5	211446066	0	805.73
S5_211752440	5	211752440	0	807.42
S5_211763116	5	211763116	0	807.76
S5_212596699	5	212596699	0	809.93
S5_212596689	5	212596689	0	810.02
S5_212596692	5	212596692	0	810.17
S5_212351248	5	212351248	0	811.22
S5_211726332	5	211726332	0	814.20
S5_211749444	5	211749444	0	814.49
S5_211749449	5	211749449	0	814.80
S5_211749446	5	211749446	0	814.99
S5_211992169	5	211992169	0	816.94
S5_214287723	5	214287723	0	824.28
S5_214352068	5	214352068	0	825.08
S5_214352056	5	214352056	0	825.25
S5_214390012	5	214390012	0	826.70
S5_214389994	5	214389994	0	827.96
S5_214115943	5	214115943	0	830.04
S5_213588619	5	213588619	0	832.87
S5_213588627	5	213588627	0	833.04
S5_213562612	5	213562612	0	833.21
S5_213819861	5	213819861	0	835.96
S5_213819865	5	213819865	0	836.40
S5_213915806	5	213915806	0	838.70
S5_213915862	5	213915862	0	838.79
S5_214078255	5	214078255	0	842.43
S5_214536625	5	214536625	0	853.25
S5_214711518	5	214711518	0	854.27
S5_215020952	5	215020952	0	858.84
S5_215342077	5	215342077	1	864.90
S6_5160638	6	5160638	1	11.81
S6_4438290	6	4438290	0	13.27

Table A.2 continued.

S6_6271001	6	6271001	0	17.38
S6_6896580	6	6896580	0	22.04
S6_22915764	6	22915764	0	24.93
S6_22916162	6	22916162	0	25.10
S6_22908295	6	22908295	0	25.61
S6_22911180	6	22911180	0	26.06
S6_22918484	6	22918484	0	27.02
S6_8145949	6	8145949	0	31.61
S6_7125119	6	7125119	0	32.23
S6_8145860	6	8145860	0	33.16
S6_8142468	6	8142468	0	33.57
S6_8145823	6	8145823	0	33.65
S6_8270648	6	8270648	0	33.82
S6_8143156	6	8143156	0	33.99
S6_4306361	6	4306361	0	43.01
S6_4307645	6	4307645	0	45.68
S6_4175521	6	4175521	0	46.32
S6_3986806	6	3986806	0	47.04
S6_4016921	6	4016921	0	48.64
S6_3678069	6	3678069	0	49.60
S6_3678075	6	3678075	0	49.87
S6_3572577	6	3572577	0	50.22
S6_3648877	6	3648877	0	50.30
S6_3249219	6	3249219	0	52.52
S6_3054149	6	3054149	0	56.44
S6_3179905	6	3179905	0	56.52
S6_3054175	6	3054175	0	56.95
S6_2242172	6	2242172	0	58.65
S6_2428165	6	2428165	0	59.59
S6_2245495	6	2245495	0	59.67
S6_2242209	6	2242209	0	59.84
S6_2382593	6	2382593	0	60.10
S6_2043451	6	2043451	0	61.20
S6_780616	6	780616	0	62.95
S6_1340916	6	1340916	0	70.39
S6_9201595	6	9201595	0	78.44
S6_9201617	6	9201617	0	78.53
S6_9194696	6	9194696	0	79.07
S6_9200467	6	9200467	0	79.39
S6_32456764	6	32456764	0	86.38
S6_32456805	6	32456805	0	86.47
S6_25374619	6	25374619	0	91.92
S6_26150477	6	26150477	0	92.80
S6_26652231	6	26652231	0	99.29
S6_26668885	6	26668885	0	100.00
S6_25337331	6	25337331	0	101.06
S6_25337393	6	25337393	0	101.77
S6_28146622	6	28146622	0	105.12
S6_28146652	6	28146652	0	105.67
S6_28146626	6	28146626	0	105.67
S6_29010794	6	29010794	0	105.67
S6_28146634	6	28146634	0	105.67
S6_29010192	6	29010192	0	105.67
S6_28146639	6	28146639	0	105.77
S6_28146630	6	28146630	0	105.94
S6_28146628	6	28146628	0	106.37
S6_29012508	6	29012508	0	106.74
S6_36947944	6	36947944	0	110.95
S6_37026721	6	37026721	0	110.95
S6_36622354	6	36622354	0	111.12
S6_36622493	6	36622493	0	111.29
S6_36622477	6	36622477	0	111.56
S6_36622435	6	36622435	0	111.82
S6_36947989	6	36947989	0	112.16
S6_37026816	6	37026816	0	112.24
S6_36947508	6	36947508	0	112.24
S6_36623640	6	36623640	0	112.90
S6_38271750	6	38271750	0	113.10
S6_38271954	6	38271954	0	113.10
S6_38792386	6	38792386	0	113.44
S6_36624158	6	36624158	0	113.96
S6_36623652	6	36623652	0	113.96
S6_36622484	6	36622484	0	114.05
S6_38064683	6	38064683	0	114.46
S6_38064679	6	38064679	0	114.72
S6_38064792	6	38064792	0	114.80
S6_38271909	6	38271909	0	114.97
S6_39370912	6	39370912	0	115.76
S6_39370888	6	39370888	0	115.84
S6_43908560	6	43908560	0	116.37
S6_43908356	6	43908356	0	116.45
S6_43908586	6	43908586	0	116.62
S6_55601052	6	55601052	0	117.40

Table A.2 continued.

S6_60191993	6	60191993	0	117.92
S6_62735937	6	62735937	0	118.61
S6_62736093	6	62736093	0	118.61
S6_57285013	6	57285013	0	119.31
S6_56103915	6	56103915	0	119.31
S6_58455064	6	58455064	0	119.70
S6_41796789	6	41796789	0	121.09
S6_42611735	6	42611735	0	121.25
S6_40439003	6	40439003	0	121.60
S6_43908527	6	43908527	0	122.02
S6_41535598	6	41535598	0	122.19
S6_41535550	6	41535550	0	122.33
S6_44006950	6	44006950	0	122.41
S6_41535622	6	41535622	0	122.49
S6_44004076	6	44004076	0	122.49
S6_43101930	6	43101930	0	122.49
S6_43907957	6	43907957	0	122.49
S6_43907785	6	43907785	0	122.49
S6_43101957	6	43101957	0	122.49
S6_41511513	6	41511513	0	122.58
S6_44821882	6	44821882	0	122.83
S6_44821933	6	44821933	0	122.91
S6_44824453	6	44824453	0	123.00
S6_43907923	6	43907923	0	123.10
S6_42612678	6	42612678	0	123.17
S6_43908386	6	43908386	0	123.17
S6_43654985	6	43654985	0	123.34
S6_44382123	6	44382123	0	124.69
S6_60182741	6	60182741	0	125.94
S6_60193147	6	60193147	0	126.03
S6_58446322	6	58446322	0	126.46
S6_59125252	6	59125252	0	126.62
S6_61315099	6	61315099	0	126.79
S6_62187989	6	62187989	0	135.91
S6_67218872	6	67218872	0	144.30
S6_72476879	6	72476879	0	145.22
S6_70293213	6	70293213	0	145.99
S6_70012455	6	70012455	0	146.15
S6_72805005	6	72805005	0	146.49
S6_70012458	6	70012458	0	146.50
S6_74035947	6	74035947	0	153.83
S6_74460087	6	74460087	0	153.83
S6_74603965	6	74603965	0	154.16
S6_76508121	6	76508121	0	154.92
S6_74603964	6	74603964	0	155.83
S6_67218491	6	67218491	0	156.45
S6_67259903	6	67259903	0	156.87
S6_66026551	6	66026551	0	157.65
S6_78616779	6	78616779	0	159.39
S6_78616095	6	78616095	0	159.56
S6_82399280	6	82399280	0	162.03
S6_82378485	6	82378485	0	162.28
S6_82814045	6	82814045	0	163.16
S6_82764692	6	82764692	0	163.33
bZIP	3	138878960	0	168.35
S6_85519531	6	85519531	0	170.29
S6_85421549	6	85421549	0	170.37
S6_89122202	6	89122202	0	172.57
S6_89825614	6	89825614	0	173.50
S6_89465268	6	89465268	0	173.93
S6_89465267	6	89465267	0	174.10
S6_91617218	6	91617218	0	175.24
S6_94003739	6	94003739	0	183.03
S6_93784130	6	93784130	0	183.38
S6_93497902	6	93497902	0	184.62
S6_89066212	6	89066212	0	192.62
S6_89123242	6	89123242	0	193.19
S6_96553422	6	96553422	0	202.09
S6_97345192	6	97345192	0	210.88
S6_97384351	6	97384351	0	211.40
S6_97088625	6	97088625	0	211.92
S6_97108674	6	97108674	0	211.98
S6_97088627	6	97088627	0	212.08
S6_97227406	6	97227406	0	212.42
S6_97233832	6	97233832	0	212.68
S6_97382439	6	97382439	0	213.11
S6_97615124	6	97615124	0	213.71
S6_97615109	6	97615109	0	214.45
S6_97615143	6	97615143	0	215.10
S6_97615144	6	97615144	0	215.27
S6_97614649	6	97614649	0	216.09
S6_96922618	6	96922618	0	217.44
S6_96922670	6	96922670	0	217.56

Table A.2 continued.

S6_96922626	6	96922626	1	218.00
S6_103360654	6	103360654	1	232.74
S6_103360855	6	103360855	0	233.02
S6_103441010	6	103441010	0	233.18
S6_103133085	6	103133085	0	234.82
S6_103133079	6	103133079	0	234.91
S6_103140123	6	103140123	0	235.25
S6_103130468	6	103130468	0	235.85
S6_105833971	6	105833971	0	243.17
S6_106251916	6	106251916	0	246.53
S6_110291736	6	110291736	0	246.62
S6_106343222	6	106343222	0	246.96
S6_106342051	6	106342051	0	247.04
S6_106344561	6	106344561	0	247.20
S6_106468868	6	106468868	0	247.29
S6_106343142	6	106343142	0	247.37
S6_106344079	6	106344079	0	247.54
S6_106343221	6	106343221	0	247.89
S6_106342083	6	106342083	0	248.24
S6_110007831	6	110007831	0	248.50
S6_110007884	6	110007884	0	248.67
S6_110534889	6	110534889	0	248.86
S6_110578211	6	110578211	0	249.12
S6_106343899	6	106343899	0	249.29
S6_106343936	6	106343936	0	249.46
S6_110783891	6	110783891	0	250.25
S6_110783900	6	110783900	0	250.42
S6_110708883	6	110708883	0	251.38
S6_107311771	6	107311771	1	255.13
S6_113109411	6	113109411	1	268.40
S6_114056956	6	114056956	0	270.58
S6_114056965	6	114056965	0	270.67
S6_113782170	6	113782170	0	270.67
S6_114440450	6	114440450	0	270.97
S6_114374565	6	114374565	0	271.09
S6_116892795	6	116892795	0	275.96
S6_115573963	6	115573963	0	276.22
S6_117999279	6	117999279	0	278.62
S6_122585386	6	122585386	0	282.26
S6_128160499	6	128160499	0	285.17
S6_128318314	6	128318314	0	287.53
S6_128583520	6	128583520	0	289.19
S6_128735763	6	128735763	0	289.87
S6_128736209	6	128736209	0	290.03
S6_128735703	6	128735703	0	290.73
S6_128583848	6	128583848	0	294.09
S6_128583869	6	128583869	0	294.18
S6_129436252	6	129436252	0	295.15
S6_128523638	6	128523638	0	296.51
S6_128522753	6	128522753	0	297.21
S6_121374874	6	121374874	0	301.95
S6_121373717	6	121373717	0	302.21
S6_121371295	6	121371295	0	302.46
S6_120159057	6	120159057	0	303.43
S6_120159100	6	120159100	0	303.51
S6_120913231	6	120913231	0	304.04
S6_127767406	6	127767406	0	308.03
S6_127769934	6	127769934	0	308.38
S6_127894985	6	127894985	0	309.27
S6_123382009	6	123382009	0	314.41
S6_123383534	6	123383534	0	314.51
S6_123156656	6	123156656	0	315.11
S6_123156661	6	123156661	0	315.71
S6_122727992	6	122727992	1	316.32
S6_141511189	6	141511189	1	359.32
S6_141513425	6	141513425	0	359.45
S6_140908334	6	140908334	0	363.16
S6_147974960	6	147974960	0	369.45
S6_147974961	6	147974961	0	370.71
S6_146818213	6	146818213	0	389.87
S6_146817571	6	146817571	0	390.03
S6_146817031	6	146817031	0	390.29
S6_146616827	6	146616827	0	390.63
S6_146665632	6	146665632	0	391.24
S6_147346041	6	147346041	0	394.96
S6_147225115	6	147225115	0	395.65
S6_147311379	6	147311379	0	396.17
S6_147311400	6	147311400	0	396.34
S6_147311408	6	147311408	0	396.86
S6_147660550	6	147660550	0	399.77
S6_147660738	6	147660738	0	400.29
S6_147660788	6	147660788	0	400.83
S6_144125372	6	144125372	0	410.48

Table A.2 continued.

S6_144280141	6	144280141	0	410.57
S6_144125412	6	144125412	0	410.91
S6_143996179	6	143996179	0	410.99
S6_149529168	6	149529168	0	418.84
S6_149469587	6	149469587	0	418.94
S6_148961561	6	148961561	0	420.76
S6_149821655	6	149821655	0	423.05
S6_150104161	6	150104161	0	423.52
S6_150024333	6	150024333	0	423.90
S6_149834541	6	149834541	0	424.17
S6_150024428	6	150024428	0	424.59
S6_150024404	6	150024404	0	424.85
S6_148593213	6	148593213	0	426.58
S6_148777179	6	148777179	0	428.66
S6_148591482	6	148591482	0	428.92
S6_148723411	6	148723411	0	429.42
S6_148777113	6	148777113	1	429.69
S6_155436490	6	155436490	1	444.93
S6_155235957	6	155235957	0	446.29
S6_155218428	6	155218428	0	446.87
S6_155218409	6	155218409	0	446.97
S6_155241116	6	155241116	0	447.51
S6_155396990	6	155396990	0	448.78
S6_155397015	6	155397015	0	449.25
S6_155461351	6	155461351	0	450.11
S6_155453264	6	155453264	0	450.37
S6_155436487	6	155436487	0	450.83
S6_155460904	6	155460904	0	450.91
S6_155453218	6	155453218	0	451.21
S6_155627512	6	155627512	0	457.41
S6_155627528	6	155627528	0	457.50
S6_155627419	6	155627419	1	457.69
S6_161707937	6	161707937	1	471.61
S6_161707915	6	161707915	0	471.79
S6_162913790	6	162913790	0	479.00
S6_163402602	6	163402602	0	485.07
S6_163402589	6	163402589	0	485.30
S6_163421359	6	163421359	0	485.93
S6_164188220	6	164188220	0	493.72
S6_164319268	6	164319268	0	494.85
S6_164319275	6	164319275	1	494.85
S6_165540939	6	165540939	1	497.65
S6_165939582	6	165939582	0	503.32
S6_165982357	6	165982357	0	504.84
S6_165989029	6	165989029	0	505.47
S6_166054985	6	166054985	0	505.81
S6_166052049	6	166052049	1	505.89
S7_4064736	7	4064736	1	9.16
S7_4064712	7	4064712	0	9.33
S7_4066463	7	4066463	0	11.91
S7_4707096	7	4707096	0	14.73
S7_4583737	7	4583737	0	18.84
S7_4965329	7	4965329	1	24.50
S7_7258869	7	7258869	1	29.67
S7_7619786	7	7619786	0	31.22
S7_8048336	7	8048336	0	33.69
S7_8048060	7	8048060	0	33.77
S7_8050438	7	8050438	0	34.37
S7_8050380	7	8050380	0	34.47
S7_8520111	7	8520111	0	35.61
S7_8569532	7	8569532	1	36.13
umc1066	7	10793341	1	41.14
S7_11573347	7	11573347	0	43.29
S7_13980470	7	13980470	0	49.20
S7_14606291	7	14606291	0	52.39
S7_14658428	7	14658428	0	53.14
S7_14658561	7	14658561	0	53.60
S7_14459913	7	14459913	0	54.39
S7_15470834	7	15470834	0	57.75
S7_15661023	7	15661023	0	60.35
S7_16192038	7	16192038	0	63.11
S7_15827153	7	15827153	0	63.27
S7_15827593	7	15827593	0	63.92
S7_16334066	7	16334066	0	64.43
S7_17180908	7	17180908	0	65.73
S7_15328721	7	15328721	0	66.98
S7_17479423	7	17479423	0	68.84
S7_17479398	7	17479398	0	69.02
S7_17479186	7	17479186	0	69.62
S7_18426587	7	18426587	0	72.41
S7_18626511	7	18626511	0	72.93
S7_18626469	7	18626469	0	73.26
S7_19083787	7	19083787	0	73.80

Table A.2 continued.

S7_19083788	7	19083788	0	73.89
S7_20244261	7	20244261	0	74.67
S7_20195664	7	20195664	0	74.84
S7_20620150	7	20620150	0	75.01
S7_19623809	7	19623809	0	75.43
S7_19489069	7	19489069	0	75.48
S7_20195597	7	20195597	0	75.60
S7_19623747	7	19623747	0	75.75
S7_19090196	7	19090196	0	75.84
S7_19492152	7	19492152	0	76.09
S7_19489036	7	19489036	0	76.36
S7_19353562	7	19353562	0	76.84
S7_19073167	7	19073167	0	77.04
S7_19083538	7	19083538	0	77.39
S7_19347658	7	19347658	0	77.48
S7_19354428	7	19354428	0	77.90
S7_19754152	7	19754152	0	78.24
S7_19347598	7	19347598	0	78.67
S7_19354761	7	19354761	0	79.32
S7_19354744	7	19354744	0	79.62
S7_19354760	7	19354760	0	79.71
Zeinssr12	7	20780000	0	81.05
S7_19353647	7	19353647	0	82.59
S7_19055229	7	19055229	0	82.59
S7_19353537	7	19353537	0	82.67
S7_19254855	7	19254855	0	82.84
S7_19354422	7	19354422	0	83.01
S7_19254582	7	19254582	0	83.18
S7_19354608	7	19354608	0	83.43
S7_19055692	7	19055692	0	83.69
S7_19254584	7	19254584	0	83.78
S7_25506608	7	25506608	0	89.07
S7_27418792	7	27418792	0	91.50
S7_27978520	7	27978520	0	92.05
S7_28818140	7	28818140	0	93.02
S7_28818290	7	28818290	0	93.10
S7_28818265	7	28818265	0	93.19
S7_29562531	7	29562531	0	93.70
S7_29562558	7	29562558	0	93.79
S7_29529370	7	29529370	0	93.96
S7_29655340	7	29655340	0	93.96
S7_40297967	7	40297967	0	94.65
S7_40412782	7	40412782	0	94.73
S7_40412789	7	40412789	0	94.91
S7_39873341	7	39873341	0	95.17
S7_40571160	7	40571160	0	95.26
S7_41418309	7	41418309	0	95.61
S7_39183583	7	39183583	0	96.85
S7_39183449	7	39183449	0	98.31
S7_47937537	7	47937537	0	102.18
S7_47725253	7	47725253	0	103.58
S7_47935582	7	47935582	0	104.37
S7_47599452	7	47599452	0	105.05
S7_47573709	7	47573709	0	105.35
S7_47935964	7	47935964	0	105.60
S7_47935939	7	47935939	0	106.03
S7_47935762	7	47935762	0	106.31
S7_50591521	7	50591521	0	107.92
S7_107485132	7	107485132	0	116.54
S7_107455959	7	107455959	0	116.71
S7_108845726	7	108845726	0	117.23
S7_109058159	7	109058159	0	118.57
S7_112538336	7	112538336	0	120.81
S7_115183592	7	115183592	0	123.34
S7_115285666	7	115285666	0	125.38
S7_115309191	7	115309191	0	125.38
S7_115692965	7	115692965	0	125.90
S7_116282107	7	116282107	0	126.51
S7_116282052	7	116282052	0	126.69
S7_116288791	7	116288791	0	126.92
S7_116375254	7	116375254	1	127.27
S7_125348297	7	125348297	1	147.49
S7_125102868	7	125102868	0	148.79
S7_125120093	7	125120093	0	149.18
S7_125120517	7	125120517	0	149.26
S7_125120511	7	125120511	0	149.34
S7_125320837	7	125320837	0	149.69
S7_125835677	7	125835677	0	150.64
S7_125835674	7	125835674	0	150.64
S7_126642240	7	126642240	0	153.23
S7_127719038	7	127719038	0	158.25
S7_127888863	7	127888863	0	158.99
S7_127892170	7	127892170	0	159.63

Table A.2 continued.

S7_127892165	7	127892165	1	159.93
S7_128139766	7	128139766	1	160.49
S7_128373677	7	128373677	0	160.75
S7_128117012	7	128117012	0	160.91
S7_130878006	7	130878006	1	172.01
S7_130245646	7	130245646	1	173.44
S7_130241616	7	130241616	0	175.01
S7_130250861	7	130250861	0	176.27
S7_130249426	7	130249426	0	178.59
S7_130249423	7	130249423	0	178.77
S7_132590140	7	132590140	0	189.18
S7_132590204	7	132590204	0	189.51
S7_133047544	7	133047544	0	193.42
S7_132922333	7	132922333	0	193.94
S7_132922867	7	132922867	0	194.20
S7_137693966	7	137693966	0	202.05
S7_137543753	7	137543753	0	202.14
S7_137521204	7	137521204	0	202.14
S7_137694015	7	137694015	0	202.14
S7_137521198	7	137521198	0	202.31
S7_137540093	7	137540093	0	202.48
S7_137543775	7	137543775	0	202.82
S7_137702729	7	137702729	0	203.39
S7_137532688	7	137532688	0	204.78
S7_135572602	7	135572602	0	210.84
S7_138617419	7	138617419	0	220.97
S7_138377280	7	138377280	0	222.35
S7_138372007	7	138372007	0	222.58
S7_138372014	7	138372014	0	222.75
S7_138371971	7	138371971	0	223.28
S7_141677912	7	141677912	0	231.92
S7_141677939	7	141677939	0	232.27
S7_141808597	7	141808597	0	235.71
S7_141826929	7	141826929	0	235.91
S7_141827004	7	141827004	0	236.09
S7_141901808	7	141901808	0	236.77
S7_142782613	7	142782613	0	237.93
S7_142783016	7	142783016	0	240.09
S7_143113582	7	143113582	0	241.93
S7_142905547	7	142905547	0	244.23
S7_142905514	7	142905514	0	244.32
S7_142998494	7	142998494	0	245.01
S7_142998501	7	142998501	0	245.26
S7_143422904	7	143422904	0	245.87
S7_143414626	7	143414626	0	245.87
S7_143410013	7	143410013	0	245.87
S7_143520572	7	143520572	0	245.87
S7_143414633	7	143414633	0	246.57
S7_143414631	7	143414631	0	246.66
S7_143414627	7	143414627	0	247.17
S7_144535532	7	144535532	0	247.94
S7_144535561	7	144535561	0	247.95
S7_145770541	7	145770541	0	249.91
S7_145770550	7	145770550	0	250.24
S7_145741935	7	145741935	0	250.85
S7_145729058	7	145729058	0	254.92
S7_145388227	7	145388227	0	256.71
S7_145596886	7	145596886	0	257.07
S7_145683441	7	145683441	0	257.25
S7_145383696	7	145383696	0	257.42
S7_145217722	7	145217722	0	257.85
S7_146118852	7	146118852	0	260.00
S7_145095073	7	145095073	0	260.97
S7_146245198	7	146245198	0	264.26
S7_146244014	7	146244014	0	264.68
S7_146596969	7	146596969	0	265.93
S7_146597906	7	146597906	0	266.90
S7_146484925	7	146484925	0	267.08
S7_146464787	7	146464787	0	267.25
S7_146597917	7	146597917	0	268.09
S5_62188638	5	62188638	0	268.44
S7_147119409	7	147119409	0	269.50
S7_147207183	7	147207183	0	269.92
S7_147207181	7	147207181	0	270.06
S7_147338907	7	147338907	0	270.83
S7_147339502	7	147339502	0	271.07
S7_147384716	7	147384716	0	272.25
S7_147381407	7	147381407	0	272.54
S7_147528237	7	147528237	0	272.97
S7_147385128	7	147385128	0	273.14
S7_147424534	7	147424534	0	273.24
S7_147417991	7	147417991	0	273.39
S7_147418015	7	147418015	0	273.48

Table A.2 continued.

S7_147582156	7	147582156	0	274.26
S7_148599260	7	148599260	0	276.62
S7_148730620	7	148730620	0	276.70
S7_148599297	7	148599297	0	276.78
S7_148592460	7	148592460	0	277.03
S7_148599174	7	148599174	0	277.13
S7_149912851	7	149912851	0	278.56
S7_149718842	7	149718842	0	279.18
S7_149755377	7	149755377	0	279.38
S7_149718792	7	149718792	0	279.48
S7_149754654	7	149754654	0	279.66
S7_149718723	7	149718723	0	280.21
S7_149852693	7	149852693	0	280.95
S7_148850993	7	148850993	0	281.41
S7_151085466	7	151085466	0	282.95
S7_150042450	7	150042450	0	283.65
S7_150041360	7	150041360	0	283.65
S7_150041531	7	150041531	0	283.65
S7_150041397	7	150041397	0	284.16
S7_150041861	7	150041861	0	284.89
S7_150874362	7	150874362	0	285.42
S7_150874224	7	150874224	0	285.42
S7_150959775	7	150959775	0	286.11
S7_150959776	7	150959776	0	286.47
S7_150888700	7	150888700	0	286.88
S7_150959771	7	150959771	0	286.97
S8_149424999	8	149424999	0	290.81
S7_152064379	7	152064379	0	295.00
S7_153101312	7	153101312	0	298.34
S7_152962881	7	152962881	0	298.42
S7_152809996	7	152809996	0	299.03
S7_152832901	7	152832901	0	299.12
S7_154627540	7	154627540	0	305.52
S7_155562725	7	155562725	0	314.73
S7_156677022	7	156677022	0	319.18
S7_157160884	7	157160884	0	320.06
S7_156739251	7	156739251	0	323.09
S7_156668843	7	156668843	0	325.34
S7_157455781	7	157455781	0	326.78
S7_157454677	7	157454677	0	326.78
S7_157473942	7	157473942	0	328.42
S7_157473936	7	157473936	0	328.51
S7_157473915	7	157473915	0	328.59
S7_158358696	7	158358696	0	331.84
S7_158953599	7	158953599	0	332.27
S7_158954050	7	158954050	0	332.38
S7_158754276	7	158754276	0	333.15
S7_161860837	7	161860837	0	341.87
S7_172779320	7	172779320	0	342.56
S7_161809222	7	161809222	0	342.74
S7_161860900	7	161860900	0	343.80
S7_161434915	7	161434915	0	345.13
S7_161434862	7	161434862	0	345.22
S7_161434966	7	161434966	0	345.31
S7_161252454	7	161252454	1	346.09
S7_162324268	7	162324268	1	348.51
S7_162324722	7	162324722	0	348.57
S7_162313439	7	162313439	0	348.68
S7_162309349	7	162309349	0	348.76
S7_165343309	7	165343309	0	357.97
S7_164782807	7	164782807	0	363.60
S7_164782781	7	164782781	0	364.41
S7_164782776	7	164782776	0	364.93
S7_164783654	7	164783654	0	365.28
S7_164079269	7	164079269	0	366.25
S7_164199900	7	164199900	0	366.52
S7_164070601	7	164070601	1	370.93
S7_167347764	7	167347764	1	378.32
S7_168449206	7	168449206	0	386.35
S7_168404916	7	168404916	0	386.87
S7_168407791	7	168407791	0	386.96
S7_168402399	7	168402399	0	387.39
S7_168402358	7	168402358	0	387.47
S7_168994892	7	168994892	0	390.05
S7_169267904	7	169267904	0	391.10
S7_169267626	7	169267626	0	391.27
S7_169267605	7	169267605	0	391.48
S7_169407251	7	169407251	0	391.70
S7_169262984	7	169262984	0	391.92
S7_170251026	7	170251026	0	396.94
S7_170251666	7	170251666	0	396.94
S7_170385721	7	170385721	0	397.81
S7_170396781	7	170396781	0	399.86

Table A.2 continued.

S7_170396819	7	170396819	0	399.94
S7_170433778	7	170433778	0	400.34
S7_170488727	7	170488727	0	401.06
S7_170488725	7	170488725	0	401.31
S7_170496549	7	170496549	0	401.57
S7_170652128	7	170652128	0	401.79
S7_170652115	7	170652115	0	402.25
S7_170649006	7	170649006	0	403.04
S7_170647905	7	170647905	0	403.74
S7_170652105	7	170652105	0	403.91
S7_170961614	7	170961614	0	405.16
S7_170966296	7	170966296	0	405.32
S7_170877587	7	170877587	0	405.32
S7_171035669	7	171035669	0	408.30
S7_171345923	7	171345923	0	409.22
S7_171582235	7	171582235	0	409.73
S7_171695299	7	171695299	0	410.69
S7_171694938	7	171694938	0	410.95
S7_171631639	7	171631639	0	413.32
S7_171629309	7	171629309	0	413.49
S7_171629241	7	171629241	0	413.66
S7_171695333	7	171695333	0	414.56
S7_171729229	7	171729229	0	414.87
S7_172163484	7	172163484	0	417.55
S7_172207443	7	172207443	0	417.72
S7_172158268	7	172158268	0	417.81
S7_172123622	7	172123622	0	417.98
S7_172123753	7	172123753	0	418.14
S7_172163784	7	172163784	0	418.49
S7_172163713	7	172163713	0	418.57
S7_172123769	7	172123769	0	418.91
S7_172123917	7	172123917	0	418.99
S7_172163710	7	172163710	0	419.42
S7_172209119	7	172209119	1	422.47
S7_174574666	7	174574666	1	427.80
S7_174574462	7	174574462	0	432.74
S7_174282779	7	174282779	0	434.89
S7_173818015	7	173818015	0	436.00
S7_173888764	7	173888764	0	436.37
S7_173888683	7	173888683	0	436.37
S7_173817965	7	173817965	0	436.54
S7_173817866	7	173817866	0	436.71
S7_173818252	7	173818252	0	436.97
S7_173963108	7	173963108	0	437.75
S7_173963049	7	173963049	0	438.61
S7_174093918	7	174093918	0	439.17
S7_176136828	7	176136828	1	444.48
S8_18231928	8	18231928	1	45.19
S8_18231723	8	18231723	0	45.53
S8_18450805	8	18450805	0	48.66
S8_17890174	8	17890174	0	50.66
S8_20829323	8	20829323	0	60.17
S8_20692458	8	20692458	0	60.35
S8_20692468	8	20692468	0	60.79
S8_20699610	8	20699610	0	61.07
S8_22678790	8	22678790	0	65.43
S8_28162631	8	28162631	0	70.01
S8_27642137	8	27642137	0	70.20
S8_26478711	8	26478711	0	70.37
S8_26862234	8	26862234	0	70.73
S8_26478680	8	26478680	0	71.16
S8_27116887	8	27116887	0	71.33
S8_28160359	8	28160359	0	71.50
S8_29731110	8	29731110	0	72.46
S8_34663721	8	34663721	0	73.95
S8_38412720	8	38412720	0	74.89
S8_40679523	8	40679523	0	76.02
S8_40682203	8	40682203	0	76.27
S8_65982891	8	65982891	0	76.87
S8_64418714	8	64418714	0	76.97
S8_65982392	8	65982392	0	77.30
S8_65982058	8	65982058	0	77.40
S8_65788274	8	65788274	0	77.68
S8_65788243	8	65788243	0	78.00
S8_64174106	8	64174106	0	78.09
S8_65785251	8	65785251	0	78.17
S8_65972918	8	65972918	0	78.78
S8_65974200	8	65974200	0	79.12
S8_69587937	8	69587937	0	80.01
S8_69587924	8	69587924	0	80.20
S8_69130177	8	69130177	0	80.54
S8_68307982	8	68307982	0	80.70
S8_69054564	8	69054564	0	80.80

Table A.2 continued.

S8_68939472	8	68939472	0	81.54
S8_69125527	8	69125527	0	82.20
S8_69757763	8	69757763	0	82.57
S8_71289475	8	71289475	0	82.91
S8_71060343	8	71060343	0	82.91
S8_71061831	8	71061831	0	82.91
S8_71652326	8	71652326	0	83.75
S8_75642537	8	75642537	0	85.78
S8_75642171	8	75642171	0	85.88
S8_75642471	8	75642471	0	85.97
S8_75643051	8	75643051	0	86.17
S8_75642510	8	75642510	0	86.29
S8_77253416	8	77253416	0	88.11
S8_76034481	8	76034481	0	88.36
S8_91422893	8	91422893	0	91.44
S8_91421946	8	91421946	0	91.78
S8_91422977	8	91422977	0	92.13
S8_91422962	8	91422962	0	92.21
S8_91421880	8	91421880	0	92.30
S8_101210007	8	101210007	0	97.60
S8_101409240	8	101409240	0	98.13
S8_101507124	8	101507124	0	98.29
S8_101507895	8	101507895	0	98.48
S8_101237818	8	101237818	0	101.00
S8_101177485	8	101177485	1	107.60
S8_104794572	8	104794572	1	116.57
S8_109378641	8	109378641	0	125.31
S8_109377740	8	109377740	0	126.08
S8_106854841	8	106854841	0	129.06
S8_107231695	8	107231695	0	129.79
S8_107219798	8	107219798	0	130.21
S8_108192828	8	108192828	0	131.38
S8_109672889	8	109672889	0	134.27
S8_109672882	8	109672882	0	134.37
S8_109910442	8	109910442	0	134.98
S8_110089372	8	110089372	0	135.37
S8_111195589	8	111195589	1	137.74
S8_115294227	8	115294227	1	147.90
S8_115294848	8	115294848	0	147.99
S8_118969518	8	118969518	0	157.17
S8_120875809	8	120875809	0	157.91
S8_121895690	8	121895690	0	158.51
S8_121892704	8	121892704	0	158.68
S8_121890400	8	121890400	0	159.09
S8_121895915	8	121895915	0	159.27
S8_121892698	8	121892698	0	159.51
S8_122063795	8	122063795	0	159.93
S8_119747737	8	119747737	0	161.14
S8_119735516	8	119735516	0	161.33
S8_119747349	8	119747349	0	161.54
S8_119735531	8	119735531	0	162.14
S8_118971709	8	118971709	0	163.98
S8_124644943	8	124644943	0	168.87
S8_126400632	8	126400632	0	171.25
S8_126400350	8	126400350	0	171.35
S8_129072671	8	129072671	0	172.64
S8_129313721	8	129313721	0	173.48
S8_129313720	8	129313720	0	173.56
S8_129313857	8	129313857	0	173.64
S8_128343924	8	128343924	0	174.89
S8_127580244	8	127580244	0	175.06
S8_128343894	8	128343894	0	175.15
S8_128343897	8	128343897	0	175.32
S8_126379067	8	126379067	0	175.66
S8_125739771	8	125739771	0	175.96
S8_125905876	8	125905876	0	176.39
S8_127579554	8	127579554	0	176.82
S8_128555681	8	128555681	0	176.91
S8_128555679	8	128555679	0	176.99
S8_128555677	8	128555677	0	177.27
S8_127579565	8	127579565	0	177.27
S8_128546766	8	128546766	0	177.35
S8_127624026	8	127624026	0	177.52
S8_127579388	8	127579388	0	177.52
S8_128601741	8	128601741	0	177.52
S8_128541902	8	128541902	0	177.69
S8_130729873	8	130729873	0	178.94
S8_130855335	8	130855335	0	178.94
S8_130926728	8	130926728	0	179.55
S8_131176679	8	131176679	0	179.63
S8_130405613	8	130405613	0	180.49
S8_130483976	8	130483976	0	180.50
S8_130327499	8	130327499	0	181.55

Table A.2 continued.

S8_130597548	8	130597548	0	181.72
S8_130855328	8	130855328	0	182.15
S8_131218328	8	131218328	0	183.02
S8_131176643	8	131176643	0	184.46
S8_131042410	8	131042410	0	184.46
S8_131042837	8	131042837	0	184.46
S8_132992521	8	132992521	0	185.91
S8_132372389	8	132372389	0	187.36
S8_132170809	8	132170809	0	187.53
S8_133275922	8	133275922	0	188.05
S8_133291001	8	133291001	0	188.13
S8_133276287	8	133276287	0	188.38
S8_133290997	8	133290997	0	188.70
S8_133934895	8	133934895	0	189.07
S8_133934897	8	133934897	0	189.07
S8_133935401	8	133935401	0	189.15
S8_134012422	8	134012422	0	189.76
S8_134071819	8	134071819	0	191.23
S8_134904119	8	134904119	0	192.10
S8_134845699	8	134845699	0	192.11
S8_135452583	8	135452583	0	192.36
S8_135452512	8	135452512	0	192.50
S8_135070848	8	135070848	0	192.64
S8_138648234	8	138648234	0	195.93
S8_138523546	8	138523546	0	197.91
S8_138648318	8	138648318	0	198.00
S8_139985678	8	139985678	0	199.83
S8_139345503	8	139345503	0	202.71
S8_139345509	8	139345509	0	204.78
S8_139345437	8	139345437	0	205.55
S8_139985659	8	139985659	0	206.07
S8_139890589	8	139890589	0	206.15
S8_142244214	8	142244214	0	209.28
S8_143309520	8	143309520	0	210.43
S8_142331283	8	142331283	0	211.06
S8_142237289	8	142237289	0	211.14
S8_142236863	8	142236863	0	211.22
S8_142296107	8	142296107	0	211.74
S8_144539426	8	144539426	0	213.08
S8_144702223	8	144702223	0	213.51
S8_145141103	8	145141103	0	213.77
S8_145141137	8	145141137	0	214.09
S8_145140385	8	145140385	0	214.28
S8_145140391	8	145140391	0	214.42
S8_145795246	8	145795246	0	219.19
S8_146483145	8	146483145	0	222.81
S8_146183773	8	146183773	0	225.03
S8_148482617	8	148482617	0	227.55
S8_148392965	8	148392965	0	227.84
S8_149918264	8	149918264	0	229.55
S8_150450074	8	150450074	0	229.74
S8_150560848	8	150560848	0	229.83
S8_150678166	8	150678166	0	230.08
S8_150677370	8	150677370	0	230.32
S8_151059913	8	151059913	0	230.51
S8_150801729	8	150801729	0	230.59
S8_150801761	8	150801761	0	230.59
S8_150801746	8	150801746	0	230.76
S8_150678579	8	150678579	0	230.84
S8_150561287	8	150561287	0	231.01
S8_151092595	8	151092595	0	231.18
S8_150450528	8	150450528	0	231.79
S8_150450503	8	150450503	0	231.95
S8_150362261	8	150362261	0	232.20
S8_149892844	8	149892844	0	232.46
S8_149692130	8	149692130	0	232.89
S8_149894275	8	149894275	0	233.40
S8_152591175	8	152591175	0	237.86
S8_151658081	8	151658081	0	238.31
S8_151908977	8	151908977	0	239.00
S8_154047896	8	154047896	0	240.16
S8_152967929	8	152967929	0	240.50
S8_152967919	8	152967919	0	240.58
S8_152968967	8	152968967	0	240.78
S8_154106868	8	154106868	0	241.01
S8_154399164	8	154399164	0	241.15
S8_155013102	8	155013102	0	241.45
S8_153089294	8	153089294	0	241.93
S8_155895215	8	155895215	0	242.47
S8_155901928	8	155901928	0	242.59
S8_156574997	8	156574997	0	243.65
S8_156575006	8	156575006	0	243.76
S8_156436526	8	156436526	0	244.36

Table A.2 continued.

S8_157098175	8	157098175	0	244.64
S8_156771932	8	156771932	0	245.26
S8_156729249	8	156729249	0	245.63
S8_157376443	8	157376443	0	246.39
S8_156870887	8	156870887	0	246.69
S8_158988135	8	158988135	0	248.69
S8_157958899	8	157958899	0	249.49
S8_159648455	8	159648455	0	250.99
S8_159901065	8	159901065	0	254.74
S8_160373429	8	160373429	0	258.87
S8_161626292	8	161626292	0	263.79
S8_161625711	8	161625711	0	263.96
S8_162561752	8	162561752	0	271.70
S8_162225921	8	162225921	0	279.08
S8_163968706	8	163968706	0	289.37
S8_164431138	8	164431138	0	290.89
S8_164363082	8	164363082	0	291.39
S8_164363096	8	164363096	0	291.39
S8_164183138	8	164183138	0	292.64
S8_164431163	8	164431163	0	293.89
S8_164626648	8	164626648	0	296.25
S8_164626679	8	164626679	0	296.42
S8_164767876	8	164767876	0	298.13
S8_164771751	8	164771751	0	298.40
S8_165182695	8	165182695	0	299.10
S8_165238764	8	165238764	0	299.35
S8_165715336	8	165715336	0	303.82
S8_165720997	8	165720997	0	304.56
S8_165720998	8	165720998	0	304.65
S8_165411189	8	165411189	0	308.74
S8_165976775	8	165976775	0	311.50
S8_166531806	8	166531806	0	312.06
S8_166617831	8	166617831	0	312.27
S8_166622363	8	166622363	0	312.66
S8_166621388	8	166621388	0	312.85
S8_166575828	8	166575828	0	315.18
S8_166561688	8	166561688	0	318.90
S8_166675259	8	166675259	0	322.19
S8_166793266	8	166793266	0	326.16
S8_166812577	8	166812577	0	328.01
S8_166812534	8	166812534	0	328.36
S8_166812533	8	166812533	0	328.44
S8_166812536	8	166812536	0	328.55
S8_167193161	8	167193161	0	332.49
S8_167155333	8	167155333	0	333.17
S8_167086456	8	167086456	0	333.78
S8_166974881	8	166974881	0	334.21
S8_167089671	8	167089671	0	335.53
S8_167085821	8	167085821	0	335.98
S8_167086551	8	167086551	0	336.22
S8_167128464	8	167128464	0	336.29
S8_167086561	8	167086561	0	336.29
S8_167085751	8	167085751	0	336.46
S8_167015032	8	167015032	0	336.46
S8_167089653	8	167089653	0	336.46
S8_167089682	8	167089682	0	336.72
S8_167086595	8	167086595	0	336.88
S8_167092631	8	167092631	0	337.00
S8_167086477	8	167086477	0	337.09
S8_168172557	8	168172557	0	342.39
S8_168917579	8	168917579	0	343.93
S8_169088222	8	169088222	0	345.09
S8_169070717	8	169070717	0	345.45
S8_169519156	8	169519156	0	352.43
S8_169665423	8	169665423	0	354.59
S8_169901252	8	169901252	0	358.31
S8_169692952	8	169692952	0	358.92
S8_169894678	8	169894678	0	359.35
S8_170135644	8	170135644	0	362.04
S8_171331625	8	171331625	0	367.49
S8_171181952	8	171181952	0	369.86
S8_171407304	8	171407304	0	370.12
S8_171327608	8	171327608	0	370.64
S8_171533134	8	171533134	0	371.25
S8_171542423	8	171542423	0	371.42
S8_171543949	8	171543949	0	371.85
S8_171597548	8	171597548	0	373.85
S8_170768641	8	170768641	0	378.62
S8_170352632	8	170352632	0	379.88
S8_170344574	8	170344574	0	381.33
S8_171646180	8	171646180	0	392.50
S8_171613738	8	171613738	0	399.04
S8_171712412	8	171712412	0	401.18

Table A.2 continued.

S8_171710311	8	171710311	0	401.26
S8_171877741	8	171877741	0	402.80
S8_171886056	8	171886056	0	402.97
S8_171875677	8	171875677	0	403.47
S8_171885776	8	171885776	0	403.74
S8_171984632	8	171984632	0	408.81
S8_172457717	8	172457717	0	417.79
S8_172693042	8	172693042	0	421.60
S8_172693125	8	172693125	0	421.88
S8_172702614	8	172702614	0	422.31
S8_172705830	8	172705830	0	422.51
S8_173607445	8	173607445	0	426.62
S8_173719655	8	173719655	0	426.96
S8_173713656	8	173713656	0	427.91
S8_173713608	8	173713608	0	428.26
S8_173927703	8	173927703	0	431.79
S8_173927720	8	173927720	0	431.96
S8_173923502	8	173923502	0	433.07
S8_173919972	8	173919972	0	433.49
S8_173873013	8	173873013	0	433.74
S8_173873024	8	173873024	0	433.85
S8_173873027	8	173873027	0	433.94
S8_173864683	8	173864683	0	434.46
S8_173864666	8	173864666	0	434.46
S8_173864688	8	173864688	0	434.54
S8_174213629	8	174213629	0	436.65
S8_175187517	8	175187517	0	437.21
S8_175214314	8	175214314	0	437.21
S8_175187436	8	175187436	0	437.29
S8_175062188	8	175062188	0	437.63
S8_174760922	8	174760922	0	437.72
S8_174983390	8	174983390	0	437.72
S8_174983344	8	174983344	0	437.80
S8_175069747	8	175069747	0	437.89
S8_174338374	8	174338374	0	438.23
S8_174307629	8	174307629	0	438.31
S8_174376927	8	174376927	0	438.39
S8_174273181	8	174273181	0	439.09
S8_173935679	8	173935679	0	440.24
S8_173935678	8	173935678	0	440.33
S8_173839577	8	173839577	0	442.17
S8_173409805	8	173409805	0	444.63
S8_173409556	8	173409556	0	444.88
S8_173321117	8	173321117	0	449.64
S8_173320958	8	173320958	0	449.89
S8_173276745	8	173276745	0	450.33
S8_173276770	8	173276770	0	450.57
S8_173243139	8	173243139	0	453.46
S8_173250854	8	173250854	0	453.55
S8_173081739	8	173081739	1	454.28
S9_14849373	9	14849373	1	35.84
S9_14849463	9	14849463	0	36.40
S9_14849369	9	14849369	0	36.68
S9_17638741	9	17638741	0	41.14
S9_18330715	9	18330715	0	42.12
S9_18881895	9	18881895	0	45.67
S9_18881858	9	18881858	0	46.04
S9_18883212	9	18883212	0	46.76
S9_18896533	9	18896533	0	48.20
S9_18947201	9	18947201	0	48.81
S9_20114167	9	20114167	1	53.16
S9_96718167	9	96718167	1	68.54
umc1691	9	95742200	0	77.16
umc1688	9	95739600	0	79.41
S9_26486065	9	26486065	0	88.23
S9_25925835	9	25925835	0	88.76
S9_22160887	9	22160887	0	93.78
S9_23257842	9	23257842	0	98.42
S9_23257807	9	23257807	0	98.78
S9_23326791	9	23326791	0	100.39
S9_23059488	9	23059488	0	106.61
S9_23212071	9	23212071	0	108.05
S9_23212098	9	23212098	0	108.48
S9_23455447	9	23455447	0	116.61
S9_42840859	9	42840859	0	123.79
S9_42840867	9	42840867	0	123.97
S9_43791234	9	43791234	0	125.11
S9_43792807	9	43792807	0	125.29
S9_43791221	9	43791221	0	125.37
S9_43122802	9	43122802	0	125.82
S9_26933054	9	26933054	0	132.29
S9_26933097	9	26933097	0	132.53
S9_26708199	9	26708199	0	136.38

Table A.2 continued.

S9_26801571	9	26801571	0	136.74
S9_26715220	9	26715220	0	137.28
S9_37700279	9	37700279	0	140.53
S9_41517783	9	41517783	0	140.87
S9_41354429	9	41354429	0	141.00
S9_40773438	9	40773438	0	141.12
S9_41517737	9	41517737	0	141.43
S9_77700808	9	77700808	0	148.99
S9_24458848	9	24458848	0	166.97
S9_50762998	9	50762998	0	179.83
S9_50763066	9	50763066	0	181.08
S9_45294620	9	45294620	0	188.45
S9_25928567	9	25928567	0	194.63
S9_47064183	9	47064183	0	198.12
S9_60085719	9	60085719	0	209.61
S9_64028872	9	64028872	0	211.96
S9_57310208	9	57310208	0	213.55
S9_57833394	9	57833394	0	213.83
S9_55511799	9	55511799	0	214.09
S9_58142185	9	58142185	0	214.26
S9_58143264	9	58143264	0	214.69
S9_73475853	9	73475853	0	215.47
S9_73248116	9	73248116	0	215.68
S9_73475852	9	73475852	1	216.25
S9_110439097	9	110439097	1	305.47
S9_111715606	9	111715606	0	308.11
S9_111999588	9	111999588	0	308.45
S9_112333121	9	112333121	0	309.99
S9_112333136	9	112333136	0	310.21
S9_112333070	9	112333070	0	310.58
S9_112489715	9	112489715	0	310.92
S9_112489822	9	112489822	0	310.97
S9_112637767	9	112637767	0	311.04
S9_112489129	9	112489129	0	311.06
S9_113062518	9	113062518	0	311.32
S9_113061876	9	113061876	0	311.57
S9_113790848	9	113790848	0	313.41
S9_114278558	9	114278558	0	313.67
S9_114553285	9	114553285	0	313.67
S9_114551281	9	114551281	0	313.67
S9_114552618	9	114552618	0	313.67
S9_114552666	9	114552666	0	313.75
S9_114551960	9	114551960	0	313.84
S9_113803317	9	113803317	0	314.82
S9_113812482	9	113812482	0	315.07
S9_113220854	9	113220854	0	316.42
S9_113826456	9	113826456	0	317.11
S9_116263700	9	116263700	1	319.47
S9_142935696	9	142935696	1	383.85
S9_140974294	9	140974294	0	396.31
S9_140949743	9	140949743	0	397.37
S9_140974370	9	140974370	0	397.37
S9_141408265	9	141408265	0	397.37
S7_71063734	7	71063734	0	401.72
S9_139750226	9	139750226	0	403.11
S9_139188337	9	139188337	0	423.90
S9_138972913	9	138972913	0	440.20
S9_132045378	9	132045378	0	445.86
S9_130919776	9	130919776	0	448.93
S9_130860645	9	130860645	0	449.01
S9_132045364	9	132045364	0	451.69
S9_132043251	9	132043251	0	451.86
S9_138317995	9	138317995	0	457.69
S9_138770876	9	138770876	0	461.41
S9_139614576	9	139614576	0	464.63
S9_139931767	9	139931767	0	464.91
S9_140347076	9	140347076	0	466.35
S9_142053532	9	142053532	0	469.53
S9_142034452	9	142034452	0	469.94
S9_141991036	9	141991036	0	470.13
S9_141936556	9	141936556	0	470.21
S9_142034481	9	142034481	0	470.21
S9_142034498	9	142034498	0	470.39
S9_143165984	9	143165984	0	472.85
S9_146207257	9	146207257	0	478.16
S9_146208004	9	146208004	0	478.77
S9_146154415	9	146154415	0	479.11
S9_146429716	9	146429716	0	481.80
S9_146528053	9	146528053	0	481.99
S9_147152269	9	147152269	0	484.98
S9_147168195	9	147168195	0	485.32
S9_147168186	9	147168186	0	485.49
S9_148200038	9	148200038	0	491.68

Table A.2 continued.

S9_148042930	9	148042930	0	491.85
S9_149382357	9	149382357	0	494.42
S9_149382482	9	149382482	0	494.51
S9_149748025	9	149748025	0	498.05
S9_149732984	9	149732984	0	498.62
S9_149733061	9	149733061	0	499.13
S9_149668552	9	149668552	0	500.01
S9_149745455	9	149745455	0	500.88
S9_149748022	9	149748022	0	501.22
S9_149974304	9	149974304	0	501.60
S9_150091297	9	150091297	0	502.91
S9_149974300	9	149974300	0	504.16
S9_151147448	9	151147448	0	508.07
S9_151819320	9	151819320	0	514.13
S9_151665733	9	151665733	0	519.73
S9_151366213	9	151366213	0	522.69
S9_152206319	9	152206319	0	526.47
S9_152206347	9	152206347	0	527.55
S9_153186968	9	153186968	1	534.08
S9_153813719	9	153813719	1	535.60
S9_153963431	9	153963431	0	536.51
S9_154096952	9	154096952	0	537.76
S9_154097276	9	154097276	0	538.64
S9_154367166	9	154367166	0	539.42
S9_154367164	9	154367164	0	539.51
S9_154454376	9	154454376	0	541.25
S9_154545723	9	154545723	0	542.22
S9_154564934	9	154564934	0	543.19
S9_154564926	9	154564926	0	543.35
S9_154564925	9	154564925	0	543.44
S9_154602408	9	154602408	0	544.38
S9_155461585	9	155461585	1	545.74
S10_3061785	10	3061785	1	5.44
S10_2782224	10	2782224	0	12.11
S10_3192554	10	3192554	0	17.01
S10_3190756	10	3190756	0	18.68
S10_4244840	10	4244840	0	25.10
S10_4071219	10	4071219	1	29.56
S10_14908455	10	14908455	1	49.71
S10_15550752	10	15550752	0	51.16
S10_18399723	10	18399723	0	52.72
S10_20975795	10	20975795	0	54.28
S10_19578396	10	19578396	0	55.16
S10_19578398	10	19578398	0	55.42
S10_19578379	10	19578379	0	55.60
S10_58393590	10	58393590	0	58.99
S10_54172651	10	54172651	0	59.41
S10_37905072	10	37905072	0	59.84
S10_34232921	10	34232921	0	60.26
S10_34417699	10	34417699	0	60.35
S10_34232837	10	34232837	1	60.43
S10_64263603	10	64263603	1	116.29
S10_63031899	10	63031899	0	116.55
S10_65099593	10	65099593	0	116.80
S10_64263605	10	64263605	0	116.93
S10_65200157	10	65200157	0	117.00
S10_70537698	10	70537698	0	117.42
S10_69009209	10	69009209	0	117.42
S10_70537696	10	70537696	0	117.42
S10_71465829	10	71465829	0	117.59
S10_72773150	10	72773150	0	118.37
S10_72808622	10	72808622	0	118.37
S10_72773239	10	72773239	0	118.46
S10_77187331	10	77187331	0	121.03
S10_88269452	10	88269452	0	129.46
S10_95894707	10	95894707	0	134.76
S10_96009375	10	96009375	0	135.01
S10_96322233	10	96322233	1	135.35
S10_94588863	10	94588863	1	138.58
S10_94434886	10	94434886	0	138.58
S10_93684902	10	93684902	0	138.66
S10_93685010	10	93685010	0	138.83
S10_93685004	10	93685004	0	138.83
S10_93685031	10	93685031	0	138.92
S10_94589703	10	94589703	0	139.35
S10_94589814	10	94589814	0	139.43
S10_92133856	10	92133856	0	140.03
S10_93396330	10	93396330	1	140.70
S10_99996946	10	99996946	1	152.98
S10_108119107	10	108119107	0	158.32
S10_108358796	10	108358796	0	158.79
S10_109345925	10	109345925	0	161.77
S10_106248373	10	106248373	0	165.13

Table A.2 continued.

S10_106433049	10	106433049	0	165.39
S10_106313759	10	106313759	1	165.39
S10_110584060	10	110584060	1	173.33
S10_110584061	10	110584061	0	173.42
S10_111776537	10	111776537	0	175.35
S10_113167356	10	113167356	0	178.53
S10_113167355	10	113167355	0	178.85
S10_113149927	10	113149927	0	181.20
S10_113149897	10	113149897	0	181.72
S10_113302703	10	113302703	0	181.89
S10_113162901	10	113162901	0	182.06
S10_113162530	10	113162530	0	182.77
S10_113162273	10	113162273	0	183.46
S10_113710691	10	113710691	0	184.80
S10_113515725	10	113515725	0	184.97
S10_113395690	10	113395690	0	184.97
S10_113395153	10	113395153	0	185.05
S10_113164641	10	113164641	0	185.22
S10_114524424	10	114524424	0	185.65
S10_115611550	10	115611550	0	186.34
S10_114771692	10	114771692	0	186.34
S10_115146777	10	115146777	0	186.34
S10_115146786	10	115146786	0	186.77
S10_112991004	10	112991004	0	188.21
S10_112938235	10	112938235	0	188.30
S10_110925611	10	110925611	0	189.08
S10_110925610	10	110925610	0	189.43
S10_111288191	10	111288191	0	189.60
S10_112205816	10	112205816	0	189.79
S10_108377085	10	108377085	0	192.89
S10_109133308	10	109133308	0	193.64
S10_109133250	10	109133250	0	193.84
S10_108999791	10	108999791	0	194.03
S10_112203949	10	112203949	0	194.26
S10_109252708	10	109252708	0	194.75
S10_115518459	10	115518459	0	196.43
S10_115518456	10	115518456	0	196.51
S10_115518429	10	115518429	0	197.11
S10_117620900	10	117620900	0	197.37
S10_117028387	10	117028387	0	197.80
S10_116865684	10	116865684	0	198.54
S10_116865651	10	116865651	0	198.78
S10_116865693	10	116865693	0	198.78
S10_116976974	10	116976974	0	198.78
S10_116894888	10	116894888	0	198.78
S10_117792260	10	117792260	0	199.14
S10_117029105	10	117029105	0	199.14
S10_117475123	10	117475123	0	199.31
S10_117559577	10	117559577	0	199.31
S10_118699049	10	118699049	0	200.12
S10_118699037	10	118699037	0	200.21
S10_118511147	10	118511147	0	200.38
S10_120535589	10	120535589	0	201.21
S10_120822262	10	120822262	0	201.25
S10_120535141	10	120535141	0	201.27
S10_120822285	10	120822285	0	201.42
S10_119959098	10	119959098	0	201.51
S10_119959081	10	119959081	0	201.51
S10_120793812	10	120793812	0	201.51
S10_119959028	10	119959028	0	201.51
S10_120828153	10	120828153	0	201.51
S10_119759296	10	119759296	0	201.67
S10_119092820	10	119092820	0	201.85
S10_118990949	10	118990949	0	202.52
S10_120793777	10	120793777	0	205.73
S10_120535156	10	120535156	0	206.88
S10_120944091	10	120944091	0	207.31
S10_120671401	10	120671401	0	207.66
S10_120220348	10	120220348	0	207.83
S10_120219818	10	120219818	0	207.91
S10_120117473	10	120117473	0	207.91
S10_118963244	10	118963244	0	208.43
S10_116893466	10	116893466	0	209.30
S10_117792251	10	117792251	0	209.39
S10_123811289	10	123811289	0	211.84
S10_123811287	10	123811287	0	212.01
S10_123576509	10	123576509	0	212.18
S10_124069055	10	124069055	0	212.43
S10_124064686	10	124064686	0	212.52
S10_124323860	10	124323860	0	213.13
S10_125541348	10	125541348	0	214.06
S10_125197581	10	125197581	0	214.23
S10_125204987	10	125204987	0	214.23

Table A.2 continued.

S10_125108843	10	125108843	0	214.23
S10_125197624	10	125197624	0	214.23
S10_125848260	10	125848260	0	214.23
S10_124523644	10	124523644	0	214.48
S10_125125400	10	125125400	0	214.74
S10_125214364	10	125214364	0	214.82
S10_125214335	10	125214335	0	214.92
S10_126490323	10	126490323	0	224.28
S10_126567358	10	126567358	0	224.75
S10_126307442	10	126307442	0	234.17
S10_126307632	10	126307632	0	234.35
S10_127594835	10	127594835	0	236.34
S10_127594788	10	127594788	0	236.56
S10_129524926	10	129524926	0	242.59
S10_129586266	10	129586266	0	242.68
S10_129586050	10	129586050	0	242.77
S10_129706462	10	129706462	0	243.16
S10_129706458	10	129706458	0	243.41
S10_129586336	10	129586336	0	243.85
S10_129586342	10	129586342	0	244.02
S10_130302279	10	130302279	0	244.70
S10_130698604	10	130698604	0	244.98
S10_130722433	10	130722433	0	245.67
S10_130449647	10	130449647	0	247.41
S10_130278991	10	130278991	0	247.84
S10_130278664	10	130278664	0	248.01
S10_130279128	10	130279128	0	248.10
S10_131105191	10	131105191	0	249.66
S10_131900350	10	131900350	0	250.51
S10_131900325	10	131900325	0	250.77
S10_131879919	10	131879919	0	251.73
S10_131879898	10	131879898	0	252.71
S10_132321828	10	132321828	0	253.69
S10_132321909	10	132321909	0	253.86
S10_132321177	10	132321177	0	254.65
S10_132239378	10	132239378	0	256.90
S10_132322849	10	132322849	0	257.34
S10_132883280	10	132883280	0	259.82
S10_132612571	10	132612571	0	259.92
S10_132620492	10	132620492	0	260.18
S10_132543042	10	132543042	0	261.53
S10_132612598	10	132612598	0	262.14
S10_132883281	10	132883281	0	263.17
S10_132971664	10	132971664	0	265.32
S10_132559869	10	132559869	0	269.04
S10_133303718	10	133303718	0	271.40
S10_133303703	10	133303703	0	272.10
S10_133553296	10	133553296	0	273.06
S10_134584970	10	134584970	0	275.63
S10_134458990	10	134458990	0	277.17
S10_134413689	10	134413689	0	277.96
S10_134413629	10	134413629	0	277.96
S10_134399594	10	134399594	0	278.77
S10_134399688	10	134399688	0	279.34
S10_134583652	10	134583652	0	279.87
S10_134583645	10	134583645	0	280.17
S10_134992067	10	134992067	0	285.47
S10_134992039	10	134992039	0	288.39
S10_135684540	10	135684540	0	289.66
S10_135684539	10	135684539	0	289.75
S10_135683489	10	135683489	0	290.09
S10_135610496	10	135610496	0	290.25
S10_136007578	10	136007578	0	292.19
S10_136007575	10	136007575	0	292.37
S10_135911707	10	135911707	0	292.69
S10_135678936	10	135678936	0	293.39
S10_135679316	10	135679316	0	293.56
S10_135686336	10	135686336	0	293.56
S10_136707303	10	136707303	0	296.57
S10_137205381	10	137205381	0	298.30
S10_136963205	10	136963205	0	298.48
S10_136963180	10	136963180	0	298.57
S10_136939382	10	136939382	0	298.74
S10_136958504	10	136958504	0	298.74
S10_137354255	10	137354255	0	300.89
S10_137354245	10	137354245	0	301.07
S10_137479788	10	137479788	0	307.55
S10_137673469	10	137673469	0	311.67
S10_138090831	10	138090831	0	314.76
S10_138048243	10	138048243	0	317.68
S10_138042686	10	138042686	0	318.38
S10_138042676	10	138042676	0	318.55
S10_137673499	10	137673499	0	319.55

Table A.2 continued.

S10_137673394	10	137673394	0	319.98
S10_138018820	10	138018820	0	320.95
S10_138018807	10	138018807	0	321.23
S10_138027275	10	138027275	0	321.55
S10_138027274	10	138027274	0	321.64
S10_138048481	10	138048481	0	322.31
S10_138091681	10	138091681	0	322.40
S10_138210389	10	138210389	0	323.63
S10_138674199	10	138674199	0	327.17
S10_138674216	10	138674216	0	327.57
S10_139839860	10	139839860	0	330.67
S10_139977418	10	139977418	0	333.24
S10_139977207	10	139977207	0	333.24
S10_139879515	10	139879515	0	333.95
S10_140051120	10	140051120	0	335.26
S10_140308575	10	140308575	0	337.98
S10_140496731	10	140496731	0	338.58
S10_140496525	10	140496525	0	339.19
S10_141008782	10	141008782	0	340.83
S10_141008792	10	141008792	0	341.17
S10_140969421	10	140969421	0	342.33
S10_140971447	10	140971447	0	342.67
S10_140984674	10	140984674	0	343.92
S10_141231934	10	141231934	0	344.17
S10_141231996	10	141231996	0	344.52
S10_142509948	10	142509948	0	352.42
S10_142715776	10	142715776	0	354.67
S10_142505992	10	142505992	0	356.81
S10_142369516	10	142369516	0	357.95
S10_142398201	10	142398201	0	358.05
S10_142397175	10	142397175	0	358.13
S10_142397301	10	142397301	1	359.10
S10_147125905	10	147125905	1	367.90
S10_147706534	10	147706534	0	373.95
S10_147727010	10	147727010	0	374.47
S10_147588997	10	147588997	0	375.89
S10_147845503	10	147845503	0	378.29
S10_147151500	10	147151500	1	378.29
S10_148970904	10	148970904	1	381.67
S10_149093129	10	149093129	0	384.98
S10_149029526	10	149029526	0	388.88
S10_149309491	10	149309491	0	389.19
S10_149309490	10	149309490	0	389.27
S10_149558048	10	149558048	0	389.36
S10_149097821	10	149097821	0	390.05
S10_149597506	10	149597506	0	390.30
S10_149811649	10	149811649	0	390.57